Aalto University
School of Chemical
Technology

School of Chemical Technology

Master's Programme in Chemical, Biochemical and Materials Engineering

DEVELOPMENT OF MICROCRYSTALLINE CELLULOSE PRODUCTION

Master's thesis for the degree of Master of Science in Technology

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Abstract of master's thesis

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Title of thesis Development of microcrystalline cellulose production

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Thesis advisor(s) / Thesis examiner(s) M. Sc. Kari Vanhatalo and M. Sc. Taina Lintunen

Date 14.11.2016 **Number of pages** 81 **Language** English

Abstract

The effects of water circulation in production of microcrystalline cellulose are not clearly defined in previous literature. This work aims to study the effects of returning the used hydrolysis filtrate back to reactor in production of microcrystalline cellulose with low consistency sulfuric acid. To measure the effects on fiber and filtrate properties, series of six consecutive batch hydrolysis were produced with three raw materials. The filtrate, referred as hydrolysate, from previous hydrolysis was used as reaction medium in following batch. One additional hydrolysis series was also produced in which the hydrolysate was only partially returned to next hydrolysis. In total, seven series of six consecutive batch hydrolysis were produced with different loading of sulfuric acid and different share of hydrolysate returned to reactor.

This process simulated the recycling of hydrolysate in a continuous process. The hydrolysate was analyzed for its chemical composition: sugar and organic acid concentrations were measured in an effort to estimate further uses of hydrolysate. Fiber fraction was evaluated to study the qualities of the produced microcrystalline cellulose and whether the hydrolysis succeeded sufficiently in the treatments.

The characters of both the hydrolysate and microcrystalline cellulose mainly depended on the raw material and the intensity of the treatment they were subjected to. The combined severity factor can be effectively used to estimate the ideal range of treatment intensities. To some extent, the intensities can be manipulated by changing the amount of hydrolysate stream returned to reactor. This intensity change is mainly driven by modification of pH level. The hydrolysate from previous hydrolysis increases the treatment intensity compared to similar hydrolysis with only water and acid, but it is not clear whether it is the produced organic acids or the sulfuric acid residues that have the effect. A product from continuous process with hydrolysate recycling has lower ISO-brightness, but this should be easily adjusted by further processing.

Keywords Microcrystalline cellulose, cellulose, hydrolysis, dilute acid, sulfuric acid

Tekijä Uula Toikka

Työn nimi Mikrokiteisen selluloosan tuotantoprosessin kehittäminen

Laitos Puunjalostustekniikan laitos

Työn valvoja Professori Olli Dahl

Työn ohjaaja(t)/Työn tarkastaja(t) DI Kari Vanhatalo ja DI Taina Lintunen

Päivämäärä 14.11.2016 **Sivumäärä** 81 **Kieli** englanti

Tiivistelmä

Kirjallisuudessa ei ole käsitelty mikrokiteisen selluloosan valmistusta sellaisessa järjestelmässä, missä hydrolyysissa käytetty neste palautetaan reaktoriin. Tämä työ pyrkii selvittämään, miten hydrolyysistä saatavan filtraatin palauttaminen reaktoriin vaikuttaa mikrokiteisen selluloosan valmistukseen kun katalyyttinä käytetään matalan pitoisuuden rikkihappoa. Työssä tehtiin kuuden perättäisen hydrolyysikäsittelyn koesarjoja, millä mitattiin filtraatin kierrätyksen vaikutuksia tuotetun kuitumateriaalin ja filtraatin laatuun. Filtraatti hydrolyysistä, jäljempänä hydrolysaatti, käytettiin seuraavan hydrolyysin reaktionesteenä. Lisäksi yhdessä koesarjassa palautettiin vain osa tuotetusta hydrolysaatista reaktoriin. Yhteensä työssä tehtiin seitsemän kuuden keiton sarjaa käyttäen kolmea eri raaka-ainetta erilaisilla haponlisäysmenetelmillä ja erilaisilla reaktoriin palautetun hydrolysaatin määrillä.

Käytetty prosessi simuloi hydrolysaatin kierrättämistä jatkuvassa prosessissa. Hydrolysaatista mitattiin sokeri- ja happoanalyysit, minkä perusteella arvioitiin hydrolysaatin jatkokäyttömahdollisuuksia. Tuotetusta kuitujakeesta analysoitiin mikrokiteisen selluloosan laadun kannalta olennaisia ominaisuuksia ja näiden pohjalta arvioitiin tuotantoprosessin onnistumista.

Sekä hydrolysaatin että kuitujakeen laatuun vaikutti keskeisesti käytetty raaka-aine ja käsittelyn intensiteetti. Käsittelyintensiteettiä voidaan tehokkaasti arvioida yhdistetyllä intensiteettitekijällä (Combined severity factor, CSF). Kontrolloimalla kiertoon palautettavan hydrolysaatin osuutta tuotetusta hydrolysaatista, voidaan mahdollisesti säädellä käsittelyintensiteettiä jonkin verran. Tämä säätely perustuu pääasiassa pH:n muutoksiin. Edellisestä keitosta saatava hydrolysaatti voimistaa käsittelyintensiteettiä jonkin verran, mutta ei ole selvää, onko rikkihappojäämillä vai tuotetuilla orgaanisilla hapoilla suurempi merkitys. Jatkuvasta prosessista, missä hydrolysaatti kierrätetään, saadaan alhaisemman ISO-vaaleuden mikrokiteistä selluloosaa. Todennäköisesti sokerien aiheuttama tummuminen on helposti jatkoprosessoinnilla valkaistavissa.

Avainsanat Mikrokiteinen selluloosa, selluloosa, hydrolyysi, laimea happo, rikkihappo

Foreword and acknowledgements

This work was done to produce meaningful knowledge about recycling of the hydrolysate in AaltoCell™ process and to complete studies in Aalto University's Master's Programme of Bioproduct Technology. In the process of this work, I have seen the immense amount of bioproduct expertise gathered Department of Forest Product Technology. I have gained insight knowledge on many issues and as the most important factor, the product design processes.

The exceptional work of Olli Dahl and Kari Vanhatalo have built a solid foundation for an array of further studies. In this work I build on their work and related literature knowledge. I am hoping that this work would help in the product development of process machinery involved in the production of AaltoCell™.

I thank Andritz Oy for making this thesis possible. The interest and participation of Hannu Råmark, Taina Lintunen and Pekka Tervola have produced me the framework of this work.

At Aalto University special thanks for supporting me also belong to Rita Hatakka, Risu, Marja Kärkkäinen, Tiia Juhala, Taneli Tiittanen and many others who have helped me in solving problems in the process.

Helsingissä 14.11.2016

Uula Toikka

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Abbreviations and definitions

Abbreviation	Definition
5-HMF	5-Hydroxymethylfurfural
Acid analysis total	The sum of furfural, 5-hydroxymethylfurfural, formic acid, acetic acid and levulinic acid
CHNS/O	Carbon, hydrogen, nitrogen, sulfur and oxygen analysis
Cook cycle no.	The number refers to the ordinal number of the hydrolysis in the sequence
CPMAS-NMR	Cross-polarization magic angle spinning nuclear magnetic resonance
DP	Degree of polymerization
HPAE-PAD	High-performance anion exchange chromatography with pulsed amperometric detection
HPLC	High perfomance liquid chromatography
Hydrolysate	The liquid portion of the hydrolysis product
LODP	Level-off degree of polymerization
NBSK	Northern bleached softwood kraft
Total organic acids	The sum of formic acid, acetic acid and levulinic acid
Total sugar content	The sum of glucose, arabinose, galactose, xylose and mannose
XRD	X-ray diffractometry

1. Introduction

A novel method to produce microcrystalline cellulose has been patented by Aalto University. The AaltoCell™ process, used in this work, aims to answer many issues in the production of microcrystalline cellulose: lower the acid consumption, maintain short reaction time, increase the reaction consistency and lower the usage of neutralization agent. In the process, pulp can be efficiently hydrolyzed in low consistency with dilute acid to provide a variety of specialized cellulose crystallites. (Vanhatalo et al., 2014)

The wash water consumption of the AaltoCell™ process is estimated by Vanhatalo et al. (2014) at 0.5 tons of water per ton of microcrystalline cellulose produced. Water treatment costs vary significantly and are estimated to be between 20–500 USD per ton of pulping effluent in the US (FAO, 2016). The efficiencies of water usage and treatment have significant effect on the production economy.

The integration of side stream products into pulping processes is an ongoing development towards biorefineries. Biorefineries aim to produce fuels, power and chemicals from biomass. In a similar development, added value from pulping processes is sought also by incorporating fiber refining as a side stream to the main product line. This added value through line integration could improve the efficiency of microcrystalline cellulose production significantly (Vanhatalo et al., 2014). The water circulation effects on fiber and hydrolysate properties will be important in estimating the water economy of both the line integrates and standalone factories.

The main objective of this work was to study the effects of process liquid circulation on properties of the fiber and the eluent in AaltoCell™ process. This work tries to seek whether the reuse of hydrolysate as hydrolysis medium will cause fiber property modifications or changes in fiber surface properties. Sugar degradation processes in hydrolysate recycling are also measured throughout the experiments. The acid hydrolyzation produces sugars and accompanied decomposition products such as 5-hydroxymethylfurfural. These products could add value to AaltoCell™ process if high enough consistencies are measured in the hydrolysate.

AaltoCell™ process can be used in processing many types of pulp grades; in this work microcrystalline cellulose is produced from two types of kraft pulp made of scots pine, oxygen delignified and bleached, and dissolved pulp made of eucalyptus.

New cellulose-based products are sought in the change towards more bio-based economy. Nanosized and microcrystalline cellulose could help to provide solutions to the increasing need for biomaterial usage. Both mechanical and chemical processes are involved in production of new cellulose based materials. This work relates to the processes involved in making microcrystalline cellulose and considers mechanically produced fibrillated celluloses and nanocelluloses only in their relation to microcrystalline cellulose.

In first part, the literary review sheds light on the cellulose properties in relation to hydrolysis, production routes of microcrystalline cellulose and reaction mechanisms involved in hydrolysis. The second, experimental part, presents the methods used in preparation of microcrystalline cellulose and the effects these treatments had. In latter section, the results are presented and discussed in light of previous studies.

2. Literary review

2.1. Cellulose structure in relation to microcrystalline cellulose

Microcrystalline cellulose is a highly crystalline cellulose product. As the crystalline properties share such similarities in microcrystalline cellulose and native cellulose, it is important to consider the thoroughly studied structural factors of native celluloses. The crystalline properties of cellulose products vary highly by the source species and the treatments the cellulose is subjected to (Palme et al., 2016). These crystalline properties highly affect the microcrystalline celluloses produced and the crystal lengths in cellulose are considered to relate with level-off degree of polymerization of cellulose described in latter chapters (Habibi et al., 2010). This chapter considers the cellulose crystalline properties and the effects it has on microcrystalline cellulose products.

Depending on plant material, from one third to two thirds by weight is cellulose and in tree material the cellulose content is around 40–47 wt.% (Sixta, 2006, p 21–68). Cellulose portion of the plant material can be separated by many pulping processes described thoroughly in the book by Sixta (2006). A wide array of products is made from cellulose fiber due to the good network forming capabilities and general availability.

Cellulose is an unbranched polysaccharide, formed by β-1,4-glycosidically linked D-glucopyranose units with a degree of polymerization of 2000–15000 (Ciolacu and Popa, 2010; Sixta, 2006, p. 21–68). Microcrystalline cellulose is similarly bonded but with lower degree of polymerization (FAO, 2015). The bond formation between glucose units lead to release of one water molecule and these adjacently bonded glucose units are rotated in plane 180° (Ciolacu and Popa, 2010). The cellulose chains in microcrystalline cellulose retain the glycosidic bonding and can also be seen as a polymer of cellobiose, consisting of two anhydroglucose units with opposite direction of free hydroxyl groups that form

the isotactic monomer unit in cellulose (Sixta, 2006, p. 21–68). Figure 1 shows the isotactic monomer unit and chain structure of cellulose.

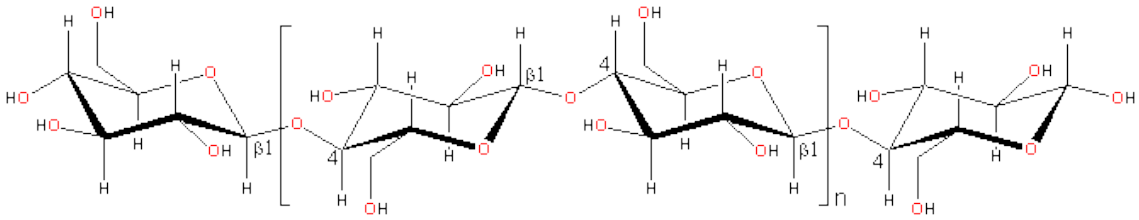


Figure 1. Structure of a cellulose chain. Cellobiose shown in brackets. (Chaplin, 2016)

In cellulose chain β-D-glucopyranose has the carbohydrate conformation of $^4C_1$: the hydrogens in axial orientation with pyranose ring and the hydroxyl groups in equatorial orientation (Ciolacu and Popa, 2010; Sixta, 2006, p. 21–68). This conformation of cellulose chain, apparently with very low free energy for a single chain allows the tight packing of cellulose chains into crystalline structures and extensive hydrogen bonding (Ciolacu and Popa, 2010; Sixta, 2006, p. 21–68; Simon et al., 1988). The hydrogen bond formation with the adjacent chains, interchain bonding, leads to formation of supramolecular crystallites (Sixta, 2006, p. 21–68; Krässig et al, 2000, p. 279–332). The intrachain bonding gives stiffness to the cellulose chain and effect the fiber network formation properties of cellulose (Shen & Gnanakaran, 2008). Alpha linkages, e.g. in starch, between glucopyranoses translate into different types of chain structures that do not allow packing into as tight crystals (Berg et al., 2002). The differences in chain packing properties are shown in Figure 2.

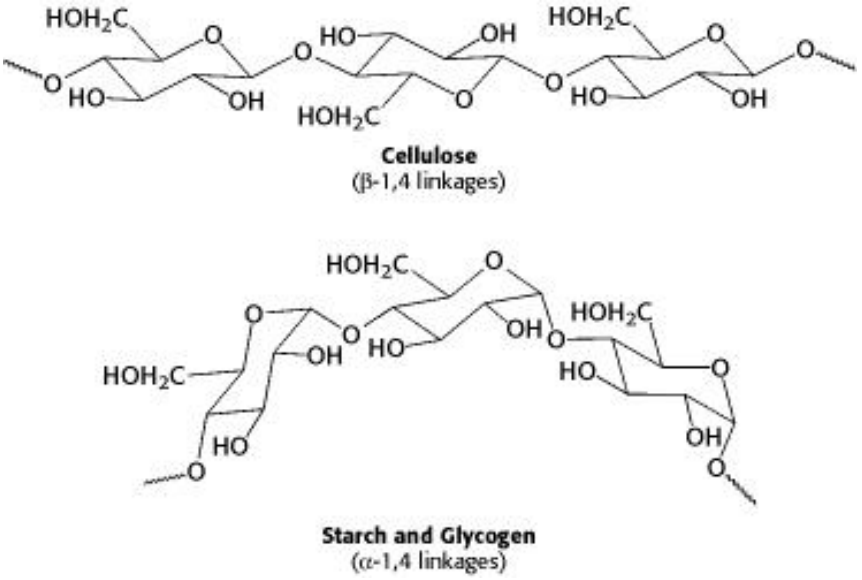


Figure 2. Bond type differences lead to different crystal packing properties (Berg et al. 2002)

Cellulose as a homopolymer incorporates domains of highly organized crystallites and less organized regions, the amorphous domains (Sixta, 2006, p. 21–68). The amorphous domains are thought to be dislocations in the fibrils caused by internal strain (Habibi et al., 2010). The crystallinity depends highly on the treatment the pulp is subjected to and the results from previous work show highly varied results depending on treatments and measurement methods: The measured crystallinity indices from raw kenaf fiber, unbleached pulp and bleached pulp were 48.2%, 68.1% and 77.3%, respectively but eucalyptus dissolving pulps only measured indices between 48% and 56% (Schild & Sixta 2011; Jonoobi et al., 2009). For materials in this study, it is expected that pine kraft pulp has crystallinity of 52%, which should rise to 63% with prolonged acid hydrolysis (Andersson et al., 2003; Liitiä et al., 2003).

The crystallinity values are mostly not comparable between studies due to differences in treatment processes and crystallinity measurement methods. The CPMAS-NMR and XRD do not produce comparable results between the methods, and it seems that

measurements produced by XRD are not comparable between studies either. In production of microcrystalline cellulose, the expected crystallinities vary highly but often the values are close to crystallinities of the cellulose that the product was made of (Virtanen et al., 2011). The effect of hydrolysis on crystallinity is open to debate but slight increase is suggested as a result of hydrolysis: Leppänen et al. (2009) observed a rise from 50% to 63% in crystallinity when kraft pulp was produced to microcrystalline cellulose.

It is suggested that moisture changes and tensile stress may cause deformations in the fibrils (Zabler et al., 2010). The crystalline regions may be separated to produce microcrystalline cellulose and produced into cellulose nanocrystals. Production of the crystalline cellulose is described in next chapter. The amorphous and crystalline regions and hydrogen bonding structures of cellulose chain are presented in Figure 3.

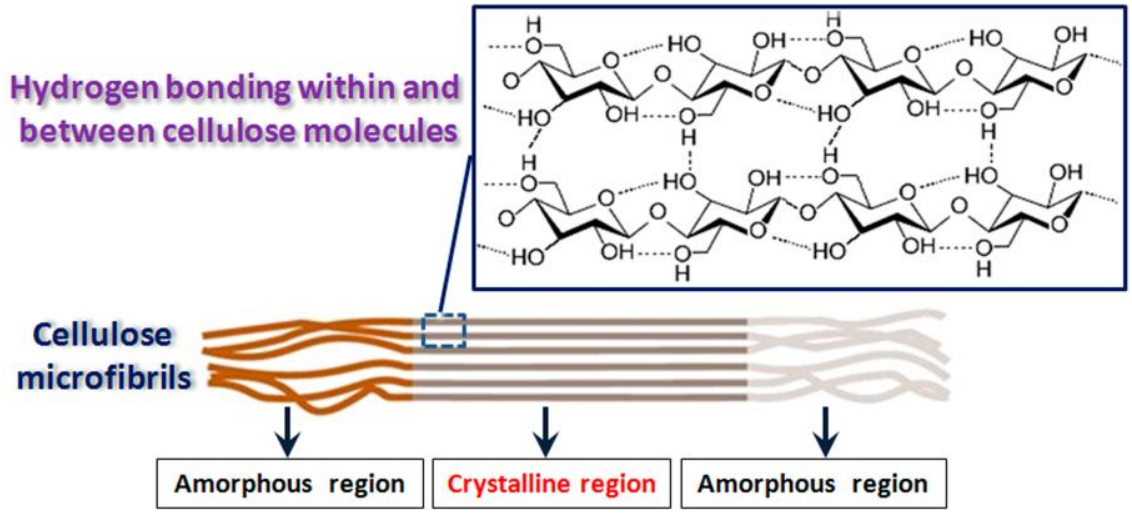


Figure 3. Schematic showing the amorphous and crystalline domains and hydrogen bonding behavior. (Zhou & Wu, 2012)

The crystalline domains in the cellulose form through a complex network of interchain and intrachain hydrogen bonding (Habibi et al., 2010). Seven different crystalline

allomorphs are recognized due to differing hydrogen bonding networks in cellulose crystals (Moon et al., 2011; Simon et al., 1988). Cellulose allomorph Iα is present mostly in algae and bacteria while the other natural form of cellulose, Iβ is present in plants (Moon et al., 2011; Habibi et al., 2010). Harsh treatment conditions or hydrothermal modification may affect the Iα/Iβ ratio in cellulose products, especially towards higher Iβ content (Matthews et al., 2012; Moon et al., 2011). In the scope of this work, cellulose is plant based and not exposed to conditions that would significantly alter the polymorphism and such considered allomorph Iβ. The other allomorphs, II, III_I, III_{II}, IV_I, IV_{II} are produced by processing the native forms or refined allomorphs, and commercial products are based also on these modified celluloses, especially cellulose II (Moon et al., 2011; Simon et al., 1988). Cellulose II is also considered the most stable arrangement of cellulose crystallites although the measurements by Kroon-Batenburg & Kroon (1997) suggest the allomorph I being an arrangement of lower energy. The mercerization and regeneration processes used to transform cellulose I to cellulose II are generally mediated in alkaline systems of over 5 N NaOH and in scope of low intensity acid treatments the cellulose is considered to retain allomorph I (Rojas, 2013; Mansikkamäki et al., 2007; Shibazaki et al., 1997). The microcrystalline cellulose currently on the market and produced in this study is of allomorph I, although new products are prepared by allomorph modifications (Rojas, 2013). Microcrystalline cellulose made in polymorph II has been suggested to alter water disintegration and pelleting properties in pharmaceutical applications and the benefits of these modifications are subjects of current studies (Krueger et al., 2014; Krueger et al., 2013). The properties of allomorphs are and their reactivity are defined elsewhere in detail e.g. in book by Ciolacu and Popa (2010).

Cellulose consists of structures that can be described in hierarchical categories: the cellulose chain, the elementary fibril, the microfibril, the macrofibril and the ultrastructure of the cell wall (Chinga-Carrasco, 2011; Sixta, 2006, p. 21–68). The

nomenclature used in defining the structures in this work is described in following paragraphs respectively. The Figure 4 shows a schematic of the fiber structure.

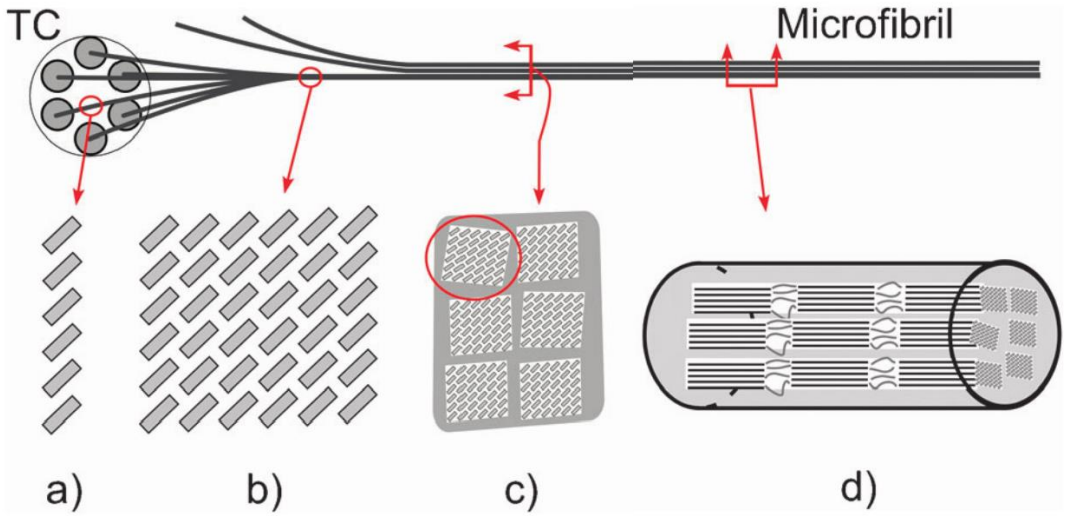


Figure 4. Schematic of the wood fiber hierarchy. a) Linear sheet of 6 individual cellulose chains synthesized by one subunit of the cellulose synthase rosette. b) Elementary fibril, product of cellulose synthase complex, 6x6 individual cellulose chains. c) Microfibril, consisting of multiple elementary fibrils. d) Cellulose chain showing sections of amorphous and crystalline domains as curved and straight respectively. Each grey box represents an individual cellulose chain. In general the microcrystalline cellulose is considered to be the crystalline parts of the chain with the amorphous parts removed. (Moon et al., 2011)

The structure of cellulose elementary fibril has been an active topic, it has been suggested that the elementary structure of plant-based cellulose consists of 18–36 cellulose chains and have asymmetric cross-section width and height between 2.9–5.3 nm (Ding et al., 2014; Thomas et al., 2013; Sixta, 2006, 21–68). It is now more accepted that the form of the terminal enzyme complex that synthesizes multiple chains simultaneously defines the structure of the elementary fibril and in plants and trees this

elementary fibril in thought to be formed of 36 chains (Moon et al., 2011). Krässig et al. (2000, p. 271–332) and Chinga-Carrasco (2011) suggest that the often seen larger microfibrils are bundles of these elementary fibrils but the size of the microfibril may be dependent on cellulose source also (Sixta, 2006, 21–68). These elementary fibrils are assumed to not be a composite structure with hemicellulose present in the bonding (Ha et al., 1998).

The elementary fibrils are bound into each other by extensive hydrogen bonding network to form microfibrils (Ha et al., 1998). The microfibrils may contain one or many elementary fibrils in cross-section embedded in hemicellulose matrix (Ding et al., 2014). The treatments used to produce microcrystalline cellulose separate the matrix to some extent and leave the crystalline regions of microfibrils mostly intact. The separation of crystalline domains is decribed in the next chapter.

Ultrastructure of the cell wall consists of differently arranged composite matrices in primary and secondary wall (Sixta, 2006, 21–68). The primary cell wall has less evident arrangement and is built by depositing the cell wall composite with hemicelluloses and lignin on the middle lamella (Sixta, 2006, 21–68). More parallelly aligned cellulose chains form the reinforcement in the secondary cell wall composite (Sixta, 2006, 21–68). Secondary wall is made of two or three layers with distinctive alignment of cellulose chains (Sixta, 2006, 21–68). Although the exact structure of elementary cellulose fibril is not clear, it is suggested that the cellulose synthesizing rosettes align in the cell wall by coordination of microtubules (Ding et al., 2014). The microtubules and associated proteins may control the synthesis and direction of cellulose in linear arrays, which synthesize multiples of elementary fibrils (Ding et al., 2014).

2.2. Microcrystalline cellulose

Microcrystalline cellulose is a product that is made by low intensity acid treatment of α-cellulose that has significantly lower degree of polymerization than native cellulose (Battista & Smith, 1961). The traditional acid treatment is done at elevated temperatures, in the methods by Battista (1962) at boiling temperature or 121 °C. The hydrolysis works in large range of acid concentrations and conditions producing slightly differing products. Different production routes may also produce microcrystalline cellulose: high shear extrusion processes with reactive oxygen species can result in hydrolysis of cellulose into microcrystalline dimensions between 80–200 °C (Hanna et al., 2001; Kopesky et al., 2007). In the hydrolysis, the crystalline regions of cellulose remain mostly intact or suffer only minor surface damages and the remaining product has order of magnitude lower degree of polymerization (Rojas, 2013; Habibi et al., 2010). It is suggested that the acid can't effectively hydrolyze the highly crystalline regions of cellulose and that the amorphous domains of cellulose are more efficiently dissolved by low intensity treatment (Palme et al., 2016). Highly crystalline areas less available for hydrolysis due to the tightly packed crystal structure that inhibits water and hydrogen ion penetration into the crystallites (Kupiainen, 2012). The definition by FAO (2015) states that microcrystalline cellulose contains at least 97 wt.% carbohydrate content of dry matter usually with a degree of polymerization of less than 400. The definition for use as food additive also states that microcrystalline cellulose should not contain more than 10 wt.% of particles with diameter less than 5 µm and restrictions on solubility, water absorption, drying loss and impurity contents (FAO, 2015; Greig, 2015). The definitions may differ slightly on the purposed use of the product, for example pharmacopoeial microcrystalline cellulose is defined as having degree of polymerization less than 350 (Thoorens et al., 2014).

Battista & Smith (1961) define the microcrystalline cellulose as mechanically disintegrated cellulose that has been treated to level-off degree of polymerization. In

acidic medium, the depolymerization starts initially at a fast rate and slows gradually to a state where the process proceeds at a very slow pace or stops, reaching the level-off degree of polymerization (Håkansson & Ahlgren, 2005; Battista et al., 1956). The initial fast hydrolysis only depolymerizes the amorphous domains and the leveling off of polymerization occurs when the remaining unhydrolyzed part consists mainly of highly crystalline regions (Palme et al., 2016). The remaining crystalline particles are generally referred as microcrystals or crystallites (Håkansson & Ahlgren, 2005).

The degree of polymerization of microcrystals depends highly on the raw material and pulping process which has been used (Håkansson & Ahlgren, 2005). From similar raw material, the degree of polymerization after similar treatment is supposed to be a function of hydrolysis harshness, though it is not exactly clear how much the materials from same source may vary in the properties of microcrystals produced in different treatments (Håkansson & Ahlgren, 2005). In length direction, LODP is usually reflecting the crystallite length measured by wide-angle x-ray scattering, but both the crystallite length and width may also increase due to hydrolysis to some degree (Leppänen et al., 2009; Ioelovich, 2016, p. 197–260). Treatments may affect the LODP also: the results of Zabler et al. (2010) suggest that changes in atmospheric humidity may affect the cellulose crystallinity and such also the LODP. The resulting effects of altering the water contents in the material are a current subject of study: an increase in LODP was measured in once-dried pulp samples by Palme et al. (2016). Long storage time may also be a factor if humidity changes are introduced. LODP values of multiple different cellulosic materials are shown in Table 1.

Table 1. Viscosities and values of LODP of fully bleached pulps from different raw materials and processes (Håkansson & Ahlgren, 2005).

Source of cellulose	Pulping method	Intrinsic viscosity (cm^3/g)	Alkali resistance (%R_{18}) SCAN-C 34:80	Intrinsic viscosity at LODP (cm^3/g)	LODP*
Cotton linters		1971	96.8	95	237
Cotton linters		1740	99.6	100	252
Aspen, birch, maple	Sulphite	1401	93.9	102	258
Birch	Prehydrolysis kraft	1001	93.5	178	497
Eucalyptus, acacia	Sulphite	501	94.7	100	252
Mixed hardwood	Prehydrolysis kraft	498	96.4	72	171
Spruce, Scotch Pine	Sulphite	1545	91.2	122	318
Spruce, Scotch Pine	Sulphite	1316	93.1	122	318
Spruce, Scotch Pine	Sulphite	661	96.1	114	294
Spruce, Scotch Pine	Sulphite	571	95.9	115	297
Pine	Sulphite	1171			

*DP values calculated using the formula $DP^{0.85} = 1.1\,[\eta]$ (Evans and Wallis 1987).

The extent to which the hydrolysis depolymerizes the cellulose is dependent also on the treatment intensity. Sixta (2006, p. 343) presents an efficient way of estimating the hydrolysis intensity by P-factor, originally by Brasch & Free (1965), a modification of Arrhenius equation, which is calculated as an integral of a function of temperature over time and shown in Equation (1) (Sixta, 2006, p. 343). This modified version of the estimation of wood chip cooking intensity, H-factor, developed by Vroom (1957) can effectively compare the treatments when similar acidities are used.

$$P = \int_{t_0}^{t} k_{rel} \cdot dt \tag{1}$$

where P P-factor

k_{rel} relative rate of acid-catalyzed hydrolysis and is temperature dependent

t time

The combined severity factor, which includes pH, temperature and residence time considers also the acidity. It may be used as an estimate of which polysaccharides will dissolve and to what extent and is another effective tool of treatment comparison (Kabel et al., 2007). The concept of combined severity factor was developed by Chum et al. 1990 and given in form shown in Equation (2) by Abatzoglou et al. (1992).

$$log(CSF) = log(t * exp(\frac{T(t) - 100}{14.75})) - pH \quad (2)$$

where CSF combined severity factor

t time of treatment,

T(t) temperature in C,

With the removal of the amorphous domains and the residues they contain, microcrystalline cellulose tends to form aggregates that are larger than the nanosized elementary fibrils (Ioelovich, 2016, p. 197–260). The commercial microcrystalline celluloses are controlled in regards to aggregation by manipulation of drying conditions to produce preferred particle size distribution (Thoorens et al., 2014). Higher acid concentrations or very intensive mechanical treatments are able to reduce the particle sizes to nanoscale (Ioelovich, 2012). Larger particle sizes in turn may be achieved by selecting specific raw material or modifying treatment intensity (Thoorens et al., 2014). The particle size distribution is a decisive factor in use: while the average particle size may be similar, the amount of large particles effect e.g. the flow properties (Thoorens et al., 2014). MCC as a material can be adapted to a wide range of different size distributions and these properties have been especially considered for use in tableting.

2.3. Production routes of microcrystalline cellulose

The process for the production of MCC was developed through the 1950's by teams that were led by Battista (Battista & Smith, 1961).The production route for level-off D.P. cellulose as described in patents by Battista & Smith (1961) and Battista (1961) uses hydrolysis of the amorphous domains in 2.5 N hydrochloric acid at boiling temperature for 15 minutes or in 0.14 N hydrochloric acid at 121 °C for one hour. The subsequent washing and optional drying continued with mechanical or high pressure disintegration produces a stable colloidal dispersion or gel. The stability is achieved primarily by means of colloidal particle size (Battista & Smith, 1961). The product is often fractionated into size classes by e.g. mechanical sifting and dried (Battista, 1962). Treatments used in dissolving the amorphous parts reduce the ash content by one order of magnitude, Battista (1962) relate this phenomena to the inorganic components retaining in amorphous cellulose and thus being dissolved in the process. The resulting cellulose product should be at least 95 wt.% glucose and preferably over 99 wt.% (Battista, 1962). This process was used to produce the patented commercial microcrystalline cellulose, Avicel® (Battista & Smith, 1961).

Most of the production routes of microcrystalline cellulose are based on the low concentration acid treatments of cellulose as described in previous paragraph. Toshkov et al. presented in their method a high yield process that decreased the aggregation by optimizing the pulping process and using 1% sulfuric acid solution as hydrolyzing medium (Toshkov et al., 1976).

Delong et al. (1990; 1986; 1983; 1981) and Ha & Landi (1998) have based their suggested production routes on steam explosion. Methods by Delong et al. aim to produce significant amount of sidestream products such as hemicelluloses, fractionated lignin, cellulose based glucose and cellulose with related derivatives such as microcrystalline

cellulose. The invention of Ha & Landi (1998) was an improvement on pharmaceutical or food processing use properties of microcrystalline cellulose: using optimized steam explosion parameters with using hydrophilic attrition aid such as carboxymethylcellulose to prevent aggregate formation in effort to produce more uniform and stable colloid.

The improvements that are made into the production often change the material properties importantly, such as altering the pelleting, aggregation, flow or mouthfeel properties. Different grades of microcrystalline cellulose products are offered mainly differing in size deviation, moisture content and additives; FMC Biopolymer describes properties of 14 differing grades of Avicel® (Avicel, 2016). The main applications of the products are in pharmaceutical pelleting and food processing.

2.4. Acid hydrolysis of cellulose

Cellulose hydrolysis is the process of depolymerizing the cellulose into the glucose constituents (Kupiainen, 2012). Many methods have been studied over time for this saccharification of cellulose chains (Liu et al., 2016). Hydrolysis in concentrated or dilute acids have been a thorough subject of study for a long time and use of acids in breakage of glycosidic and other bonds in lignocellulosic material was already studied in the 19th century (Ioelovich, 2012; Habibi et al., 2010). As stated before, cellulose chain is tightly packed into a crystalline structure with extensive hydrogen bonding, what makes the cellulose microfibrils very resistant to acid treatment in comparison with the hemicelluloses (Wijaya et al., 2014).Also the cellulose crystalline and amorphous regions have differing availabilities to hydrolysis: the glucosidic bonds in amorphous domains break 1000–5000 faster than glucose bonds in crystalline areas (Krässig et al., 2000, p. 281).

The acid hydrolysis mechanism of glucosidic links is assumed similar in all regions of cellulose, and such the description of hydrolysis mechanism in complete saccharification also apply in partial depolymerization processes (Krässig et al., 2000, p. 281; Kupiainen, 2012). The Figure 5 shows the hydrolysis of the glycosidic bond.

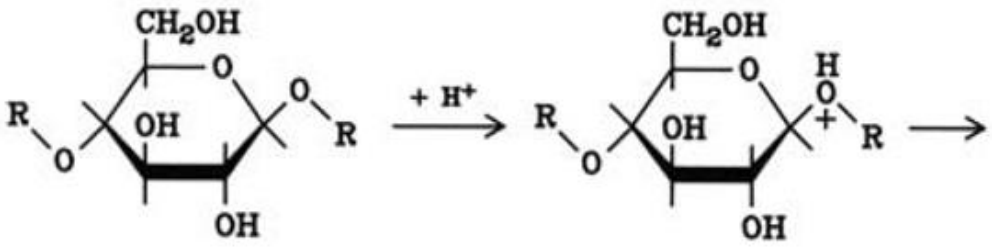


Figure 5. Hydrolysis of glycosidic bonds. (Krässig et al, 2000, p. 279–332)

Acid hydrolysis is used indifferent concentrations and reaction conditions to produce fiber products and sugar fractions. Most widely used treatment is the pretreatment of lignocellulose with dilute sulphuric acid to separate hemicelluloses and cellulignin (Dussan et al., 2014). The raw materials, process intensities and material hysteresis effect the properties of the products strongly: for example higher rates of acid hydrolysis were measured in dried Kraft pulp than in never-dried samples (Palme et al., 2016). This phenomena of varying product properties are related to crystallinity and LODP properties described earlier and hydrolysis product size deviance varies by properties of the raw material and the process involved (Moon et al., 2011). Relation of temperature to the hydrolysis intensity is shown in Figure 6, the levelling off of polymerization is seen in the stabilization of the degree of polymerization to certain level.

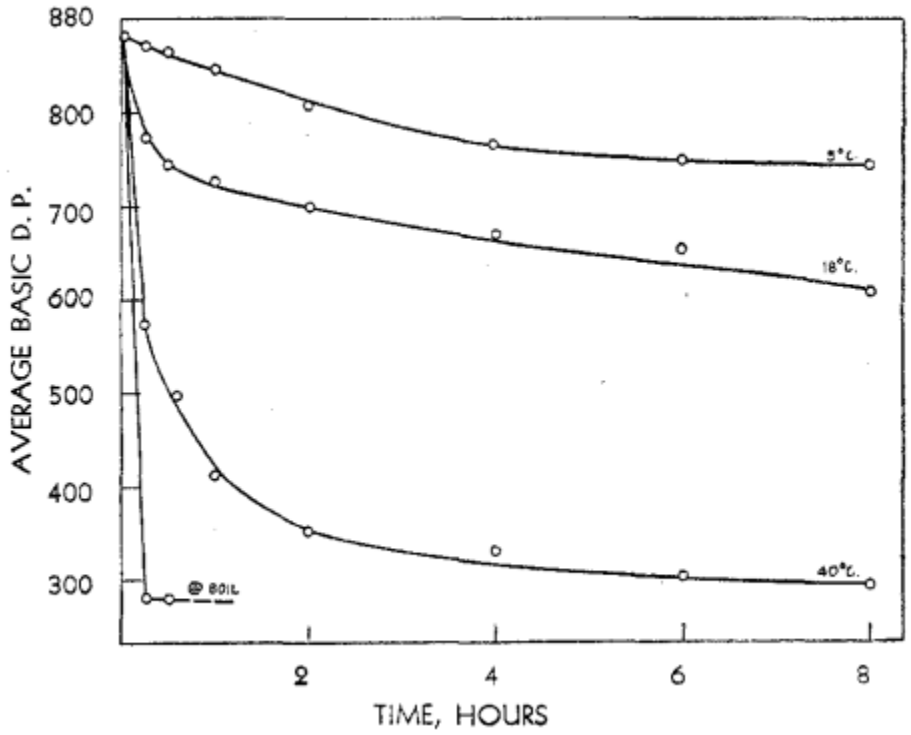


Figure 6. The effect of temperature to the hydrolysis of cotton linters in 5 N HCl. (Battista 1950)

At high acid concentrations, over 63 wt.% of sulfuric acid also the crystalline structure is infiltrated and native celluloses are hydrolyzed by replacement of glycosidic bond by hydroxyl group. Production of nanocrystalline cellulose is typically done in this level of acid concentration (Peng et al., 2011). Microcrystalline cellulose treated with 65–70 wt.% sulfuric acid will generally regenerate into low-molecular flocs of cellulose II polymorph, but when the concentration of sulfuric acid is higher than this, in most situations the cellulose will only regenerate into amorphous cellulose (Xiang et al., 2003a; Ioelovich, 2012). The higher the intensity of the treatment, the higher the share of glucose separated from fibers. Hydrolysis is driven by low pH also in temperatures under 100 C, but heat above this temperature significantly increases the hydrolysis rate (Krässig et al, 2000, p. 279–332). A pretreatment in room temperature in very high acid concentrations, will affect the subsequent hydrolysis also: the relation between acid concentration in pretreatment and undissolved glucan is shown in Figure 7.

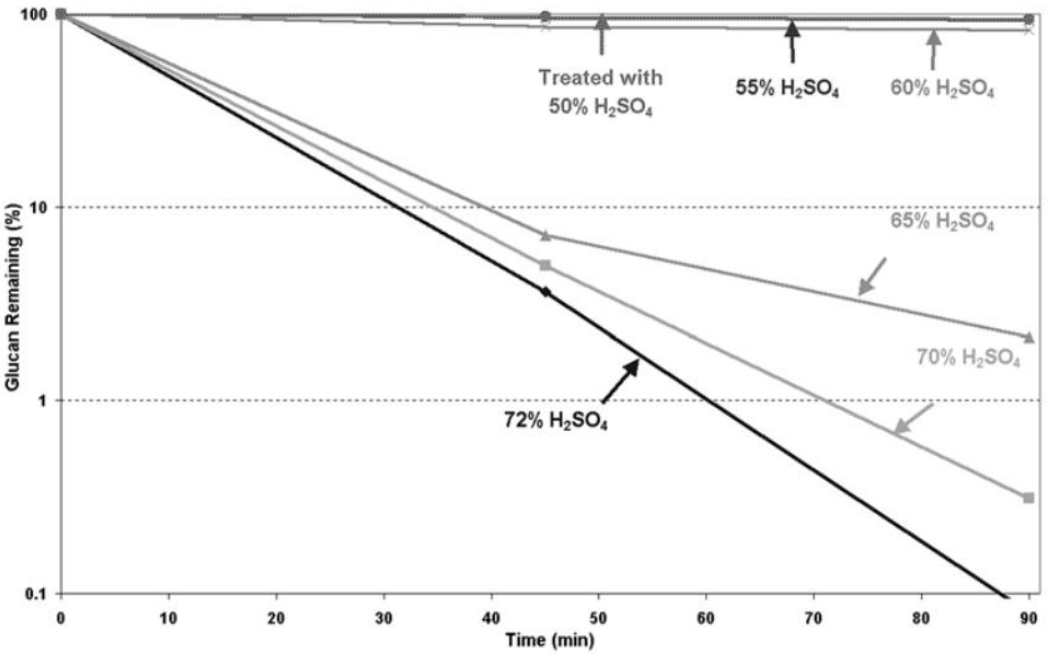


Figure 7. Dependency of undissolved glucan from sulfuric acid concentration used in pretreatment. The high concentration pretreatments lasted 4 h in 25 °C and the hydrolysis was done at 120 °C and 4% sulfuric acid. (Xiang et al., 2003b)

Acid treatments produce fermentation inhibiting substances, such as 5-hydroxymethylfurfural, furfural, other sugar degradation products and soluble phenolic lignin residues (Wijaya et al., 2014). Hydrolysis processes in general are started with processed plant material with low amounts of hemicelluloses and lignin, in some cases to improve fermentation into alcohol (Moon et al., 2011). The glucose degradation processes are described in next chapter.

2.5. Glucose decomposition processes

The decomposition of glucose in acidic medium is a complex phenomenon involving multiple reactions and multiple products; in the scope of this study the most important reaction is the dehydration of hexoses and pentoses into 5-hydroxymethylfurfural and

furfural respectively (Kupiainen, 2012; Sumerskii et al., 2010). This process is followed by further decomposition into organic acids and condensation reactions into humin-like substances (Sumerskii et al., 2010). The formation of 5-hydroxymethylfurfural, furfural and organic acids are of particular interest as they are valuable chemicals if produced in reasonable quantities. Although valuable, the furan compounds and humins act as fermentation inhibitors, so for fermentation purposes their quantities must be controlled (Sumerskii et al., 2010). The simplified decomposition routes of biomass in acidic medium with products of interest are described in Figure 8.

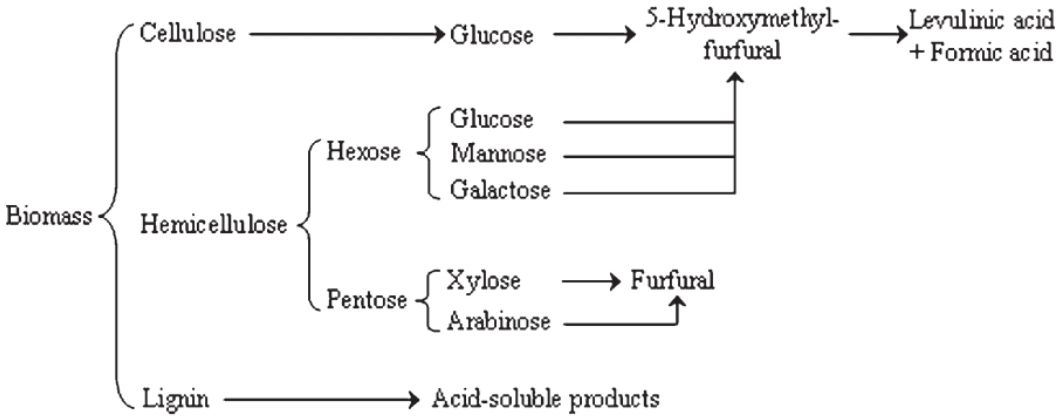


Figure 8. Simplified degradation process of biomass in acidic medium considering most interesting products (Girisuta et al., 2006).

The side product formation of humin-like substances is considered mostly as loss, as condensation into humins lower the amount of desired products. Sumerskii et al. 2010 measured the produced humin fractions from all tested monosaccharides between 21–29 wt.% of initial sugar masses in conditions of 0.5 wt.% sulphuric acid, 2 h retention time and 175–180 C. Another results suggest maximum values for the insoluble humin formation of 20% of the decomposition products and this magnitude of yield loss can be considered significant (Baugh & McCarthy, 1988). Lower humin formation was perceived in degradation of 5-hydroxymethylfurfural; it is a suggested that the quick decomposition of 5-hydroxymethylfurfural into organic acids in dilute acidic medium at

high heat resulted in low humin consistencies (Sumerskii et al., 2010). 5-hydroxymethylfurfural is anyhow a component of condensates and the amounts of humin formation affect levulinic acid yields. Increasing the heat affects the reaction rate significantly: at hydrolysis temperatures considered the 5-hydroxymethylfurfural could decompose into organic acids in minutes (Girisuta et al., 2006). Formation of humins is much dependent on the process conditions, e.g. the size of the humins based on 5-hydroxymethylfurfural was modified by an order of magnitude with the change in reaction conditions (Tsilomeleikis et al., 2016). The humin production is expected to limit yields of 5-hydroxymethylfurfural, furfural and organic acids in this study and as humins are condensate combinations of multiple types it is hard to conceive a market use for these type of low-consistency impure materials.

The reversion reactions of glucose are not expected to occur in the process involved in microcrystalline cellulose production (Kupiainen, 2012). Isomerization of hexoses may affect the monosaccharide consistencies to some level in high heat treatments, but as the produced humin fractions are similar for all hexoses, the isomerization is not expected to modify the yields of preferred products too much (Sumerskii et al., 2010; Usuki et al., 2007).

2.6. Hydrolysate products

Acid hydrolysis produces a filtrate that is a complex mixture of dissolved components and their derivatives. This hydrolysate consistency depends on the raw material: in addition to cellulose related products, hemicellulose and lignin contents produce a variety of derivatives (Girisuta et al., 2006). Some of the derived products could be separated and sold if high enough quantities are produced, e.g. levulinic acid is considered a platform chemical that could substitute oil-based chemicals (Bozell et al., 2000). The monosaccharides in hydrolysate can further form furan compounds e.g. furfural, which act as fermentation inhibitors (Sumerskii et al., 2010). The inhibitors can

cause trouble in a setting that aims to produce alcohols. Simplified reaction scheme of the degradation products is shown in Figure 9.

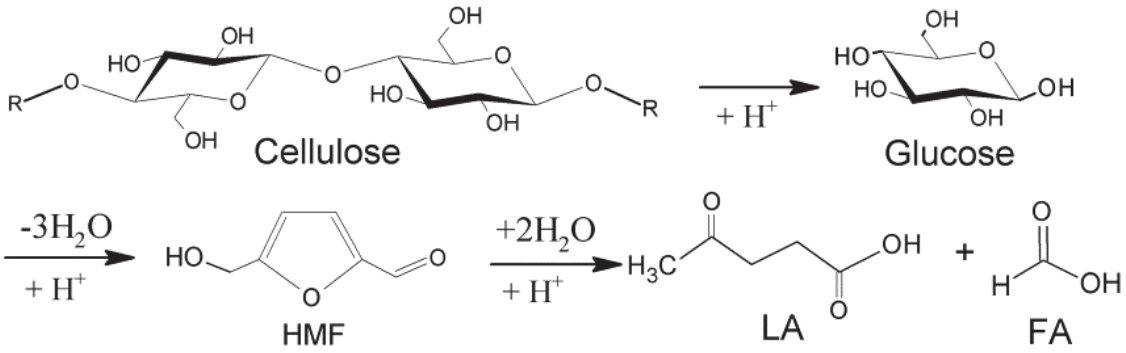


Figure 9. Decomposition of cellulose to glucose and further degradation to 5-hydroxymethylfurfural. HMF is 5-hydroxymethylfurfural, LA is levulinic acid and FA is formic acid. (Kupiainen, 2012).

The hydrolysate product composition is controlled by many factors, some of which are not clearly understood. The pH effects the hydrolysis products strongly: the decomposition of glucose is driven by acid catalysis when the pH is lower than 2 and base catalyzed when pH is higher than 2.5 (Baugh & McCarthy, 1988). The glucose decomposition rate is also more pronounced with increasing acidity at pH lower than 2 (Xiang et al., 2004). Product consistencies for the soluble formation from glucose seem to be controlled by temperature, time and pH (Baugh & McCarthy, 1988). The product composition for these products can be estimated with severity factor if raw material and process conditions are consistent. The models used in estimating the produced solubles can still only be used for a limited range of applications, and extrapolation outside model ranges is not applicable (Kupiainen, 2012).

5-hydroxymethylfurfural is formed as a product of glucose decomposition (Kupiainen, 2012). It is considered a potentially valuable platform for the production of green chemicals such as levulinic acid derivatives (Morone et al., 2015). 5-

hydroxymethylfurfural readily decomposes into levulinic and formic acids in conditions used in cellulose acid hydrolysis (Girisuta et al., 2006). In addition to this reaction route, the 5-hydroxymethylfurfural also is involved in formation of soluble crosspolymers and forementioned humins (Mukherjeev et al., 2015). The decomposition rate is highly affected by temperature, in 181 C, 5-hydroxymethylfurfural was completely decomposed in 10 minutes (Girisuta et al., 2006). The proportional consistency of formic and levulinic acid from 1:1 may be changed by reaction conditions in which the organic acids may further degrade (Girisuta et al., 2006). Temperature changes may drive the differences in relative consistencies of levulinic acid to formic acid (Kupiainen, 2012). Best processes in 5-hydroxymethylfurfural refining could already be competitive with their oil-based competitors: production of paraxylene terepthalic acid could be viable if cheap sugar resources are available (Mukherjeev et al., 2015).

Hemicelluloses degrade to form furfural, which is considered a valuable chemical. Furfural production from materials that have low hemicellulose content, e.g. kraft pulp, is not viable. Untreated wood or pre-hyrolysis liquor may be used as an input for furfural production in biorefinery (Baktash et al., 2015). Furfural can also be considered as green platform chemical and a precursor for levulinic acid production (Morone et al., 2015).

Organic acids may become important part of the biorefinery process concept, if the production process in acid treatments allow efficient separation of acids. Lignin and hemicellulose contents in raw material affect the organic acid formation: in high intensity treatment lignin decreased and hemicellulose increased the organic acids fraction in the hydrolysate (Yoon et al., 2014). In dilute acid hydrolysis, even if high total production of organic acids is achieved, the separation of them from dilute stream is often problematic (Mukherjeev et al., 2015).

Levulinic acid is expected to become a platform chemical for production of many valuable chemicals (Mukherjeev et al., 2015). The hindrances of process implementations are often stated as issues in process maturities, such as product separation issues and high chemical costs and because of these issues the commercial production has not catched wind (Morone et al., 2015). Price of the levulinic acid production has dropped significantly in 10 years and new processes should change the variety of affordable uses for levulinic acid (Mukherjeev et al., 2015).

Formic acid and acetic acid, significant sideproducts in hydrolysate, are common industry chemicals (Gao et al., 2013). The production of these kind of acids could be integrated into the biorefinery concepts.

2.7. Kinetic models

Most kinetic models for dilute acid hydrolosis of cellulose are based on pseudo-first-order reaction model drafted by Saeman (1945). The early models assumed that cellulose hydrolysis produces glucose, and glucose further degrades to other products, as shown in Figure 10.

$$\text{Cellulose} \xrightarrow{k_1} \text{Glucose} \xrightarrow{k_2} \text{Degradation products}$$

Figure 10. Reaction pathway of early cellulose hydrolysis models (Sribala & Vinu, 2014).

Multiple improvements have since been made to account for glucose derivatives, different availabilities of cellulose, humin and oligomer formation and reversible reactions (Kupiainen, 2012). In general, the Arrhenius type equations used in the modeling have given reasonable results. An example of modified Arrhenius equation by

Kupiainen (2012) is shown in Equation (3). Factors may be included or omitted in other models, but the general form used is similar. The base term has been left out from Equation (3) as the low concentration of hydroxyl ions has negligible effects in pH less than 2.

$$k = \left(k_{H2O} + k_{H+} C_{H+}\right) e^{-\frac{E_a}{RT}} \quad (3)$$

where

	k	the rate constant for cellulose degradation
	k_{H20}	solvent factor
	k_{H+}	acid factor
	C_{H+}	acid concentration
	E_a	activation energy,
	R	gas constant
	T	temperature.

When the limits that the cellulose crystallinity, acid soluble lignin-glucose complexes and humin formation set on the integrity of the models are taken into account, the hydrolysis model explained the product consistencies with coefficient of determination over 91% and for most of the studied conditions even better (Yan & Yang, 2014).The reaction scheme of Yan & Yang (2014) is shown in Figure 11. These results are a significant improvement in modeling efficiency and gives more accurate product consistencies than model by Girisuta et al. (2007), which included 5-hydroxymethylfurfural degradation reactions and humin formation.

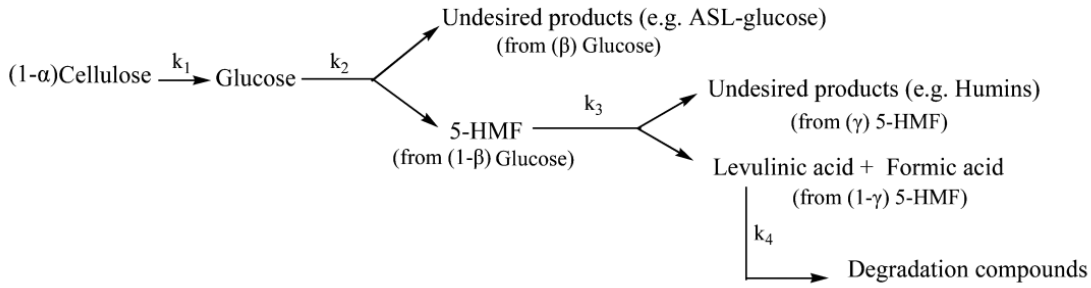


Figure 11. The reaction scheme to which Yan & Yang (2014) built their model.

The models may be used to improve the hydrolysis efficiency remarkably. Dilute acid process glucose yields in laboratory settings have been improved from 70% to 85% with the implementation of extremely dilute acid hydrolysis through modeling results (Xiang et al., 2004). These kind of developments may have made extremely low concentration acid hydrolysis an viable alternative to enzymatic route in complete hydrolysis (Gurgel et al., 2012). Many new models, such as models by Kupiainen (2012) and Yan & Yang (2014) aim to model the cellulose degradation over a large range of conditions. This approach seems to result in improving understanding of cellulose hydrolysis, but the dependencies over raw material and sensitivity to condition changes make the models not robust enough. Multiple types of models for acid hydrolysis have been developed recently, with different types of acids used (Kupiainen, 2012, Lu & Mosier, 2008), shrinking core model for cellulose hydrolysis (Wang et al., 2012) and substituted cellulose (Mu et al., 2015) to name a few. Models still need to be further refined to cater for all conditions and raw material adaptations but the models have already been beneficial in new process innovations.

2.8. Hydrolysis enhancing methods and other hydrolysis routes

In addition to acid hydrolysis, multiple other catalysts are used in the hydrolysis of cellulose. The main hydrolysis process from cellulose to glucose is often driven by acid

hydrolysis or enzymatic hydrolysis, but pretreatments with e.g. alkali or steam explosion are often used to enhance glucose yields (Rocha et al., 2015; Dussan et al., 2014). Many research lines focus on complete saccharification with fermentation to ethanol. Simultaneous saccharification and fermentation is suggested to achieve high yields and low equipment investment costs (Li et al., 2016; Ullah et al., 2014). Acid treatments are also used as pretreatments or efficiency enhancers in enzymatic hydrolysis or in novel production routes that are continuously developed, such as using gammavalerolactone as main catalyst (Ye & Chen, 2015; Han et al., 2015).

Enzymatic hydrolysis of cellulose to glucose usually aims to produce bioethanol (Taherzadeh & Karimi, 2007). Saccharification with cellulase enzymes is combined with fermentation in sequential or simultaneous production setting (Taherzadeh & Karimi, 2007). Different types of enzymes are used in combination to effectively produce glucose from the long cellulose chains (Singhania, 2009). The enzymatic hydrolysis offers an interesting route to produce similar sugar-derived chemicals that can be made with acid hydrolysis. Most of the efforts focus on producing ethanol, but for this scheme to be economically viable, the solid concentration of the process needs to be over 20% (Sotaniemi et al., 2016). The enzymatic hydrolysis may efficiently be incorporated in some biorefinery systems as multiple process developments are heading forward.

Ionic liquids have been stated as a green chemical for cellulose hydrolysis. These salts that have a melting point under 100 ºC are very expensive and near full recovery of chemicals would be necessary to keep costs manageable (Hu et al., 2015). Ionic liquids can be engineered to practically limitless forms of solvents (Hu et al., 2015).

Also, methods of hydrolysis with solid acid catalysts are being developed to overcome e.g. the trouble of separation of acid from the hydrolysate (Hu et al., 2015). Use of solid acid catalysts could solve the many problems involved in complete saccharification of

cellulose (Shen et al., 2014). Various different types of solid acids are considered: acid resins, metal oxides, H-form zeolites, heteropolyacids, functionalized silicas, supported metals, immobilized ionic liquids, carbonaceous acids and magnetic acids are all thought to be potential catalysts (Hu et al., 2015). Solid acid catalysts often are combined with pre-treatments or combined treatments with e.g. ionic liquids (Peleteiro et al 2016). The solid acid hydrolysis processes have issues that are yet to be solved e.g. obtaining sufficient contact of cellulose with the acid and separation of the solid residue from the catalyst (Parveen et al., 2016; Hu et al., 2015).

2.9. Further processing and cellulose nanoparticles

In acid hydrolysis, the intensity of the treatment effects the size of the produced particles and very often microcrystalline cellulose is used as a platform for other chemically modified cellulose nanoparticles (Brinchi et al., 2013). Nanosized particles of cellulose present highly differing properties depending on their size and related size distribution. Cellulose nanomaterials are a family of products that end in multiple uses and are made by multitude of processing routes. These nanoparticles are divided into type classes by their manufacturing route, which can be chemical or mechanical modification or the nanocellulose may be of bacterial, algal or animal origin (Lin & Dufresne, 2014). The dimensions and surface properties affect the purposed uses of nanoparticles significantly and surface functionalization further expands the proposed fields of use (Moon et al., 2011). Materials based on cellulose nanoparticles have gained significant attention in scientific study: the composites with other materials could produce very strong or biocompatible materials (Lin & Dufresne, 2014; Brinchi et al., 2013). Nanocellulose products are elaborately walked through in a review by Moon et al. (2011).

2.10. Implications of theory to this work

Most important degradation products will be accounted for in the sugar and acid measurements. Sugar degradation may lead to formation of humic substances, but as the humic substances are not considered for any use, they are accounted mainly as losses. Humic condensates could include a variety of not well known components.

Due to treatments, small changes in crystallinity should be measured, likely towards higher crystallinities. As the intensities of the treatments may change, the produced fiber, sugar and acid yields are expected to differ in different cooks. In the expected acidities the process is driven by acid-driven hydrolysis.

The intensity of the treatment should control the produced fiber properties. The results are expected to produce a better understanding for production of microcrystalline cellulose in a continuous process for certain property demands. Especially degraded sugar components and derivatives attached to fibers could deter the use of the product from many purposes if the brightness of the product is significantly lowered.

3. Experimental part

3.1. Materials and Methods

Three kinds of pulp were used in the process to produce microcrystalline cellulose:

- Dissolving pulp sheet made of eucalyptus. Sheets were cut into 3x3 cm squares before hydrolysis. Eucalyptus is referred in following results as euca.
- Fully bleached softwood kraft pulp made of pine and delivered as wet pulp. Referred in following results as kraft, bleached.
- Oxygen-delignified softwood kraft pulp made of pine with a kappa value of 22, delivered as wet pulp. Referred in results as kraft, oxygen delignified.

The pulp materials for the measurements were preferably delivered as moist pulp, as drying affects the acid hydrolysis pace (Palme et al., 2016). Other pulp properties are shown in Table 2.

Table 2. Pulp properties. NBSK is northern bleached softwood kraft.

Sample type	ISO Brightness	Limiting viscosity number [n]	Dry matter content
		ml/g	%
Dissolving pulp, eucalyptus	87.4	515.8	95
Kraft, oxygen delignified, pine	27.2	1153.5	31
NBSK, pine	85.6	953.0	35

Conductometric titration and CHNS analysis was performed for comparison of changes in fiber surface charge and sulfur content due to processing. XRD was measured to evaluate crystallinity.

In the process, 6 hydrolyses were made in a series with addition of untreated pulp material into each batch. First hydrolysis of the series were started with adding de-ionized water into reactors with pulp, subsequent hydrolyses used the hydrolysate from previous batch instead of water. For each pulp, two series were produced with different acid loading setup as described below. The hydrolysis process is described in Figure 12 on next page.

The acid load was set in two different ways:

1. 1.5 wt.% acid load on dry pulp was added in each hydrolysis. The circulation of the process water lead to increase in total H^+-ion concentration in the hydrolysis medium towards the end of the series due to accumulation of organic acids and possibly sulfuric acid residues from previous hydrolyses. In results this acid load is referred as constant.
2. Acid load was set by measuring the H^+-ion concentration in the hydrolysate and the concentration was kept same in every hydrolysis. This leads to accumulation of weak organic acids and lower share of strong sulfuric acid towards the end of the series. In results this acid loading is referred as titrated.

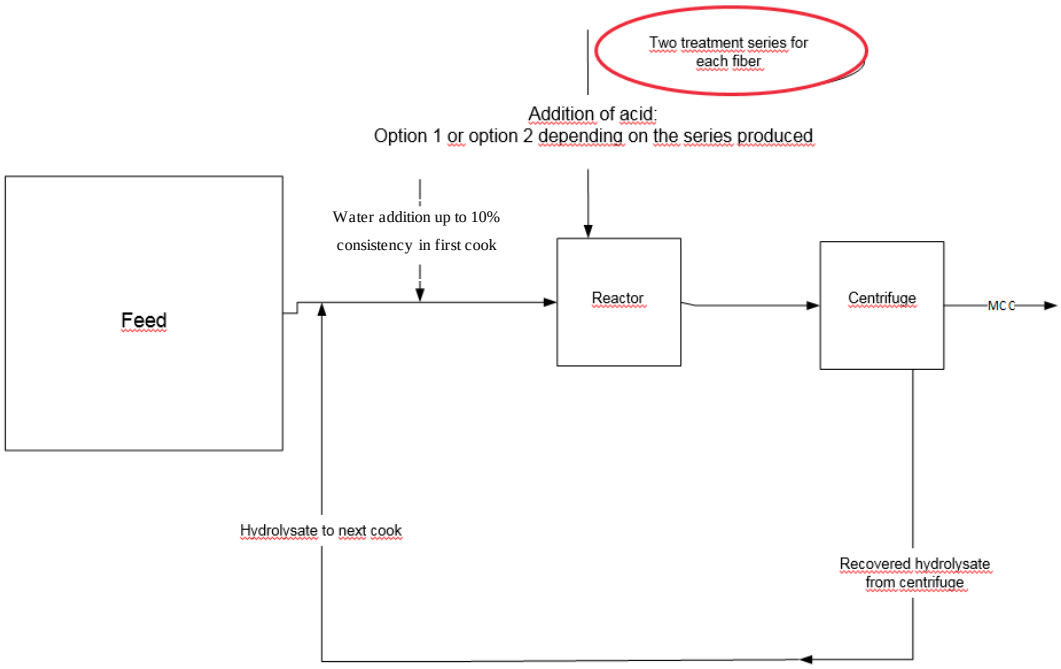


Figure 12. Diagram of the process used in hydrolyses. Option 1 is addition of 1.5 wt.% of sulfuric acid on dry fiber into each hydrolysis. Option 2 is addition of acid by measuring H^+-ion concentration and adding only the amount needed to keep the H^+-ion concentration same in each hydrolysis. Feed is either euca, kraft, bleached or kraft, oxygen delignified depending on the series produced.

In addition to six series described above, one special setting with fully bleached kraft as raw material was produced in which 35.7% of the hydrolysate produced in previous hydrolysis was returned into next batch. The rest of the hydrolysis medium liquid was taken from first wash that was made differently than in other hydrolyses: 450 ml of water was used in washing 100 g of dry pulp. This series was only done with constant sulfuric acid addition of 1.5 wt.% of acid on dry fiber in each hydrolysis. In results section, this treatment is referred to as kraft, double wash.

The hydrolyses were performed in air bath digester made by Haato Oy. The digester holds 6 autoclaves with individual volume of 2.5 liters. The digesters can be pressurized

and heated in continuous rotation with circulating air up to 180 °C. Only the pressure built up during hydrolysis was applied. The autoclaves were loaded with pulp, water and sulfuric acid in stated order. The acid was pipetted into the liquid portion. 10% pulp consistency was used in all hydrolyses.

Autoclaves were loaded in the oven and similar heating period was used for all experiments: the heat buildup up to 50 °C was unrestricted and after that the heating was slowed down to approximately 1 °C / min. The complete hydrolysis cycle took between 2h 35min and 2h 40min and the extent of hydrolysis was also followed with the H-factor shown in the process control panel of the oven.

The acid solution was centrifuged from the pulp with UPO centrifuge at 3000 rpm in a filter bag until no significant stream of hydrolysate was observed. The hydrolysates were collected: 200 ml was used in analyses and the rest was used in subsequent hydrolysis of the series.

Pulp was washed 3 times with approximately 3 l of de-ionized water and centrifuged in between each cycle. The last centrifugation was timed for a duration of 5 min and produced pulps had dry matter contents of 28–44% after centrifugation. Hydrolysis yields were determined by weighing the washed pulp, measuring the dry matter content according to SCAN C 03:78 –standard and calculating the yield with acquired wet weight of produced pulp and the dry matter content.

Fiber fraction was subjected to following measurements:

- Viscosity was measured according to SCAN C 15:99 –standard to produce estimates of degree of polymerization. For microcrystalline celluloses produced, the degree of polymerization was calculated as described in SCAN C 15:99 – standard. For untreated celluloses, the estimate of DP was calculated according to SCAN 15:88 –standard.
- Particle size distribution was measured with Malvern Mastersizer 2000. The main measurement used in evaluation was average particle size d(0.5).
- ISO brightness was measured from laboratory sheets made from 1 g of dry cellulose material. The pulp was wet disintegrated with 250 ml of de-ionized water for 2 min. Sheet was formed in Bühner funnel on 110 mm VWR filter paper no. 415. Sheets were dried by two minutes of pressing each in middle of four absorbent papers. Quick drying was performed in Lorentzen & Wettre quick dryer for 3 minutes in between 2 absorbent papers and followed by 3 minutes of drying without absorbent papers. The ISO brightness values were measured with Lorentzen & Wettre Elrepho SE070R measurement device.
- CHNS analysis was applied to test whether sulfur is collecting on the fiber surface. Test were performed with Perkin Elmer CHNS/O 2400 series II – measurement device.
- Determination of surface charge was measured by conductometric titration. The surface charge measurement with this method gives lower, more accurately fiber surface conditions describing, concentration values to strong sulfonic groups than CHNS (Lloyd & Chris, 1993). The determination was done according to SCAN CM 65:02 –standard, except washing was done as described above and stronger sodium chloride and hydrochloric acid solutions were used to produce equivalent concentrations as stated in standard. The titration was done with Metrohm 751 GPD Titrino Potentiometric Titration System. The measurements were done on raw materials and fibers produced in last hydrolysis of series with constant acid loading.

- XRD was measured to compare crystallinity changes due to the treatments. Measurements were done with Panalytical X'pert Pro –series device. The measurements were done only for raw materials and first and product from last hydrolysis of series with constant acid loading. XRD crystallinities were calculated with peak height method by Segal et al (1962).

Hydrolysate fraction was measured for following properties:

- Sugar concentration analyses were performed with Thermo Scientific Dionex HPAE-PAD –device equipped with CarboPac 20 –column against all reported standards.
- Thermo Scientific Dionex equipped with Acclaim Organic acid, OA 5 µm, 12 Å – column was used for HPLC analysis for organic acids, furfural and 5-hydroxymethylfurfural.
- Dry matter content of hydrolysates were analysed by pipetting hydrolysate into a dry matter pans with lids with known weight. After drying overnight at 105 °C the weights were recorded and reduced weight was divided by original weight to produce dry matter ratio.
- Total organic carbon analyses were performed with Shimadzu TOC-control V as the measurement device and the measurements were performed as described in EN 1484 –standard.

Evaluations of mass balance calculations were produced for series with titrated and acid loading to ensure that all material was properly accounted for in results from measurements. Calculations were produced in two ways: combining fiber yields with either produced hydrolysate dry matter content or total organic carbon analyses and comparing summed values to the amount of input into reactor. The method with hydrolysate dry matter and total organic carbon were equal as methods and only hydrolysate dry matter was used in evaluation of uncertainty factor. Equation for uncertainty factor used in evaluation of process integrity is given in Equation (4). The

mass balance equation is part of Equation (4): the part from which the absolute value is taken in calculation of uncertainty factor.

$$UF = 1 - |((YM_i + (((FDM_i - (FDM_{i-1} * (1 - FSC_{i-1}))) * 1000) * (9 * P_i/1000)))/P_i)| \quad (4)$$

where

UF	uncertainty factor
YM	yield mass in grams
FDM	filtrate dry matter share
FSC	share of liquid in hydrolysis from pulp which is constant in each series and dependent on raw material dry matter content
P	dry pulp in hydrolysis in grams
i	cook cycle number.

Similar calculation was also provided with yields, acid contents and sugar contents, but it was deemed less accurate measure as sugars continuously degrade to acids and it is very complicated to estimate how much sugars and acids are produced in a specific hydrolysis in middle of series.

All tests were done in duplicate or triplicate, except four samples in CHNS-analysis which were measured only once. For double wash series, CHNS and XRD analyses were not measured. The measurements from other series showed that no changes should be detected in these values. All reagents used were laboratory grade. Sulfuric acid was diluted to concentration of 1 M prior use. Titration of the acid contents in the hydrolysate were done with 0.1 M NaOH with Brand digital bottle-top burette. Methyl red was used as indicator under constant stirring with magnetic stirrer.

3.2. Results and discussion

3.2.1. Yields and mass balances

The mass balances showed that in general the experiments were successful. The uncertainty factor showed discrepancies more than 4% of total material only for kraft, bleached. Results from six series with three different raw materials are compared to produce comparison between raw materials and the double wash setting is compared separately to series made with same raw material, kraft, bleached. Comparison of yields from titrated and constant acid loading settings are shown in Figures 13–16. Exact values for measured points are listed in Appendices 1 and 2.

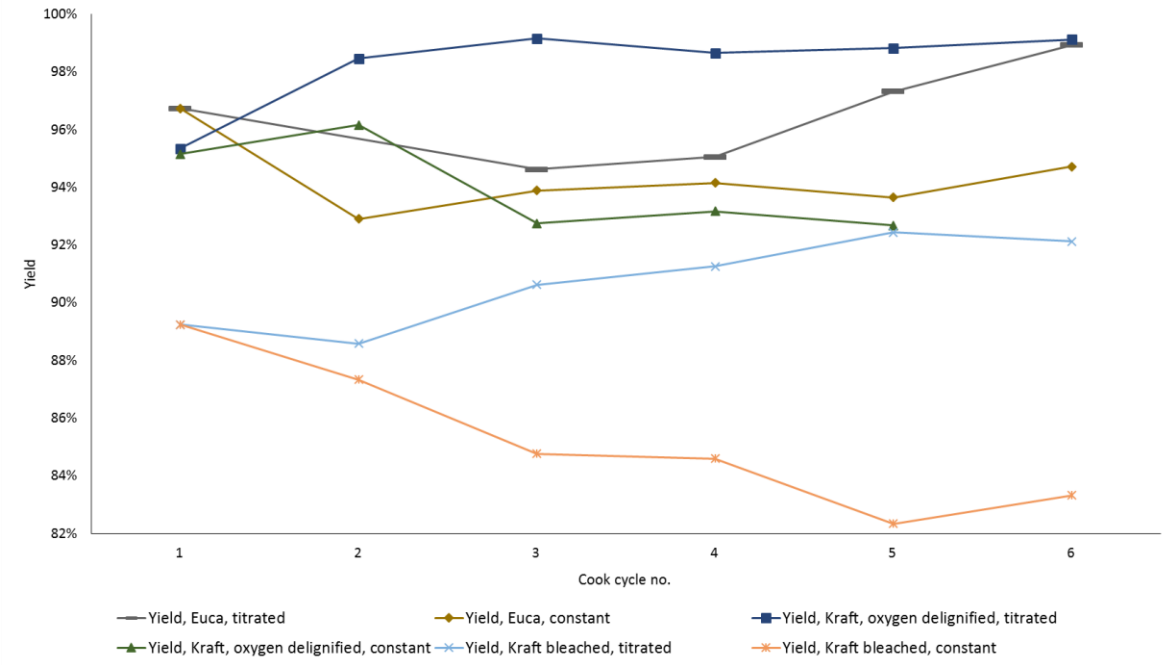
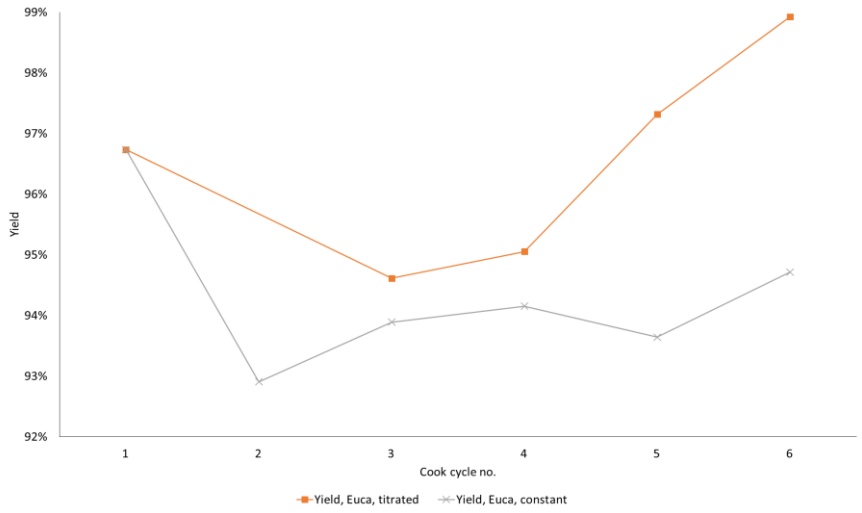


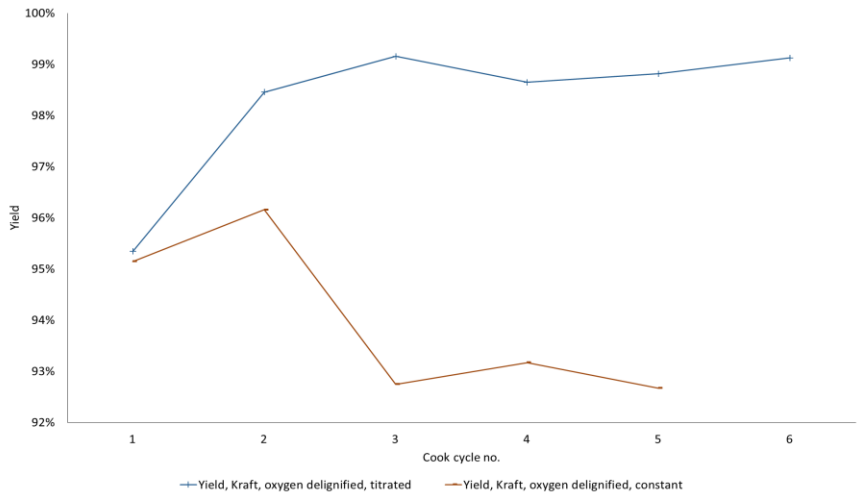
Figure 13. Yield of 6 series with titrated or constant acid load for comparison between raw materials.

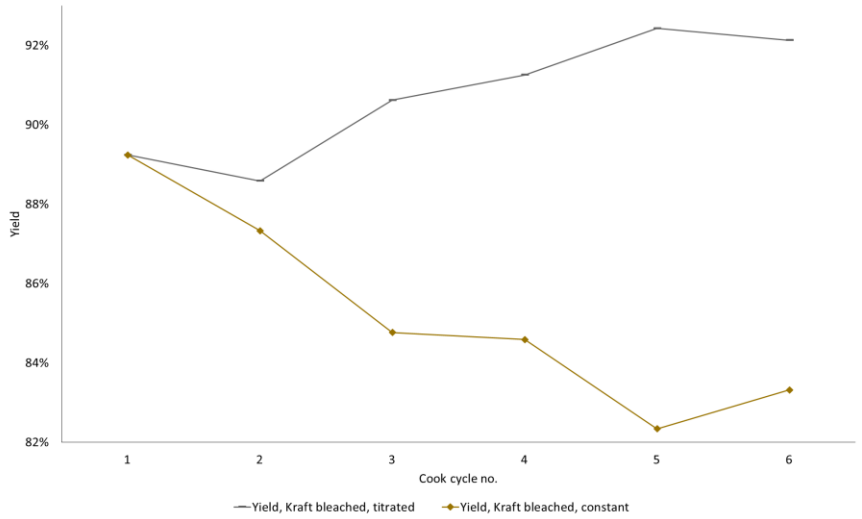
Figure 13 shows low yield values for kraft, bleached. The unexpected losses in two series made with kraft, bleached were caused by loss of very fine material in washing. The

washing losses were evaluated by reproducing two hydrolyses to compare yields with and without fiber washing. All of the mass lost was lost due to washing and the amount of mass was equal to uncertainty factor with an accuracy of two percents. The yields for kraft, bleached are in line with results produced by Vanhatalo & Dahl (2014) when washing losses are taken into account. The uncertainty factors are listed in Appendix 1. Values of yields above 90% can be possible when microcrystalline cellulose is produced from high alpha-cellulose raw material such as dissolving pulp. The results obtained still depend on the exact process settings. Results with eucalyptus were in line with process by Tomar et al. (2015).

In Figures 14–16 the separated raw material yields clearly show that the intensity of treatment changes by proceeding of different acid loading scenarios: in series with constant acid loading the intensity is increasing with proceeding cook cycles and in titrated acid loading decreasing. This shows that organic acids or residues of sulfuric acid from previous hydrolysis effects the treatment intensity, but that the organic acids are not strong enough to maintain the treatment intensities in these concentrations. Much higher concentrations of organic acids are required to successfully produce microcrystalline cellulose (Kupiainen, 2012). The increase of the intensity must be controlled in a process environment by optimization of the acid loading but the estimation of the correct amount of sulfuric acid addition can't be done by following only H^+-ion concentration. A possible solution for the optimization could be using only part of the filtrate as described in double wash scenario produced in this work.







Figures 14, 15 and 16 from top down. Comparison of yield development in series with different acid loading scenarios.

The sugar concentrations lost in washing were not considered in first round of experiments. Garlock et al. (2011) report that in their process with dilute acid, the washing produced 20 wt.% of the total glucose yield in the washing filtrate. The yield losses measured afterwards from unwashed fibers proved that up to 11.3 wt.% was lost in washing. In a factory setting, this can be recovered by using the first washing filtrate in the process. By keeping the process intensity constant, some or all of this loss can be avoided. This was shown in the results from double wash setting which used washing filtrate in hydrolysis in addition to the hydrolysate. In this series, only 35.7% of the hydrolysate stream was returned to reactor: this seemed to keep the hydrolysis intensity stable as the series was proceeding. This resulted in stabilization of sugar and acid levels after first hydrolysis to produce a fiber yield 86–89%. A comparison of double wash yields to other series produced with the same raw material are shown in Figure 17.

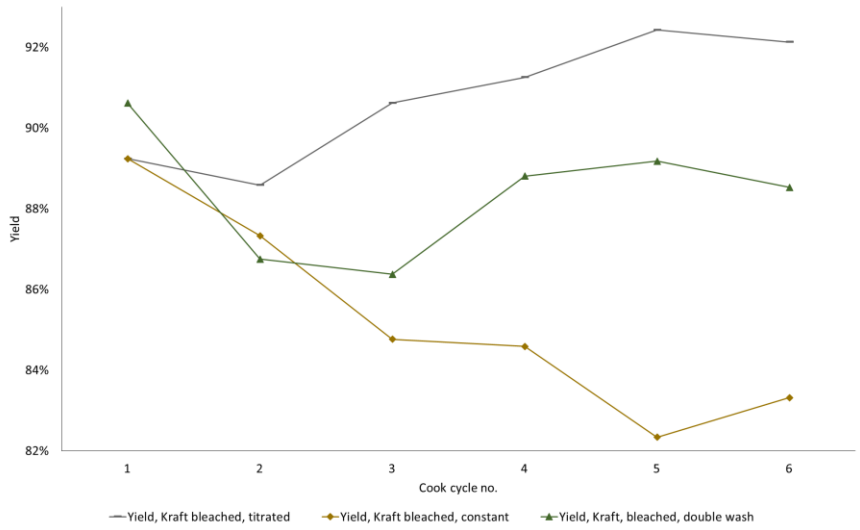


Figure 17. Comparison of yields from kraft, bleached in different treatments.

3.2.2. DP and pH and their relationship to yields

The degree of polymerization followed the expectations of plateauing at these intensities to LODP. To completely degrade the cellulose crystalline structures, orders of

magnitude higher acid concentrations should be used as described in literary review. However, the changing intensities of treatments due to variations in pH allowed the plotting of DP against pH. Other factors that would affect hydrolysis intensity were constant and only pH was changing so the treatment intensity was controlled by pH only. The pine and eucalyptus had large differences in response to treatment: the eucalyptus raw material already had significantly lower DP and this resulted in different behavior in terms of DP to pH. The relations of DP to pH are shown in Figures 18 and 19. Degree of polymerization seems to plateau at little less than 500 units in series with kraft at significantly lower levels in series made with euca.

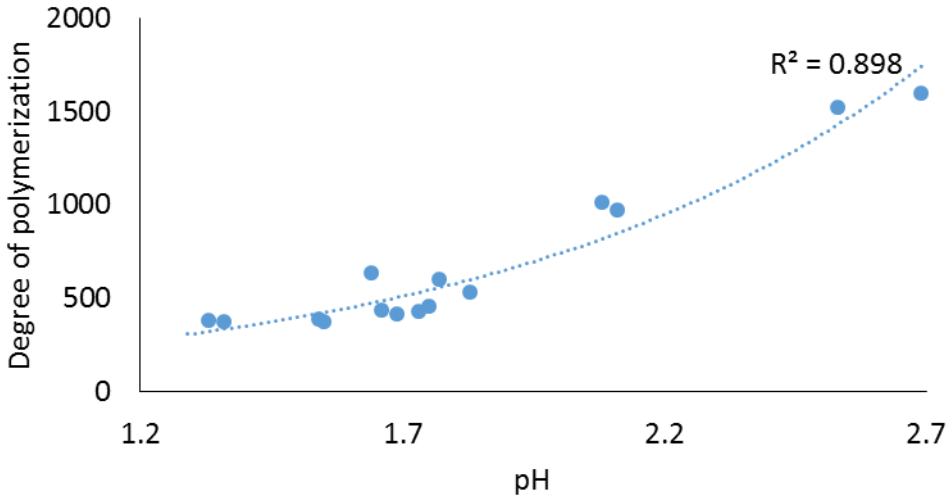


Figure 18. Relation of DP to pH in five series: kraft, bleached, titrated, kraft, bleached, constant, kraft, oxygen delignified, titrated, kraft oxygen delignified, constant and double wash. The curve is drawn for visual purposes only and equation is omitted as results from were not conclusive to produce this kind of model.

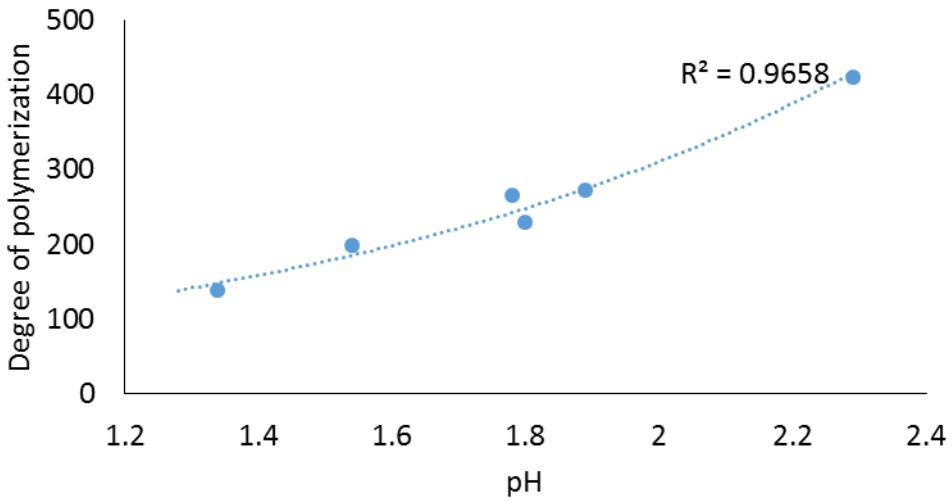


Figure 19. Relation of DP to pH in two series: euca, bleached, titrated, euca, bleached, constant. The curve is drawn for visual purposes only and equation is omitted as the study setting can't produce conclusive evidence to support this kind of a model.

LODP values seem to be little higher than what are reported by Hallac & Ragauskas (2011), Laka & Chernyavskaya (2007) and Håkansson & Ahlgren (2005) but conclusions of actual LODP are impossible to make as the treatment differs significantly from LODP measurement. The behavior in this dilute acid treatment is similar that was expected: the LODP mechanism was clearly seen in results.

The resulting yield changes from pH variations are shown in Figures 20 and 21. The results show that the controlling factor of treatment intensity was pH, as was expected. Other hydrolysis properties that would affect the combined severity factor were constant and such only pH effected the treatment intensity. The yield seemed to follow the pH in kraft, but for conclusive statements to produce models, the sample size is too small. For euca the variation was very high, even while the similar relation exists with yield and pH.

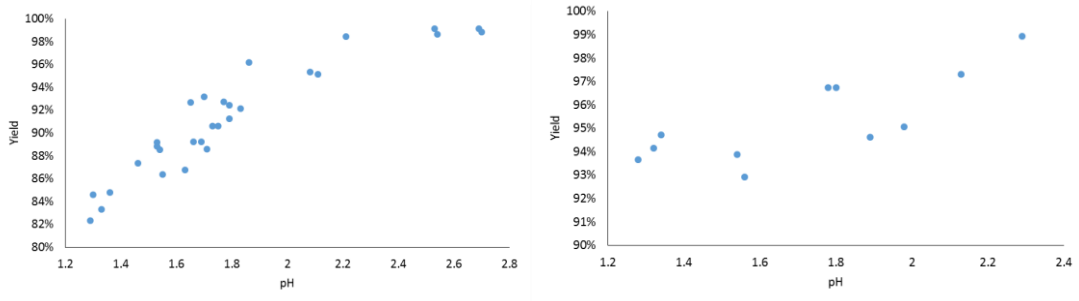


Figure 20 on left and Figure 21 on right. On left, relationship of yield to pH of five series of kraft and on right, two series of euca. Figures have differing y-axles.

The differences in treatment responses may be due to raw material differences. Most likely, at least with the yield comparisons, the dissolving pulps show higher yields due to higher alpha-cellulose content. The differences between tree species also participate to provide variation.

3.2.3. Organic matter in hydrolysate

Hydrolysate dry matter consisted of practically only carbon that is measurable in total organic carbon –measurements. The materials produced different amounts of carbon components, euca produced less as less material was expected to dissolve. Comparison of TOC to hydrolysate dry matter is expected to produce linear relationship. The evaluation of this relationship also further ensured the integrity of the results. These values also relate to intensity of the treatments, but euca as dissolving pulp is expected to produce less TOC-measurable carbon in similar treatments also. The dry matter contents produced were specific to each raw material. Different treatments also resulted in varying results with kraft, bleached, possibly due to troubles in washing process. As the behavior with these were dissimilar, the relationship between hydrolysate dry matter and TOC values is considered separately for kraft, bleached, titrated and kraft, bleached, constant. The differences in results with two series of kraft,

bleached may be due to high fine or degraded material losses in washing with kraft, bleached, constant. Results are shown in Figures 21–24.

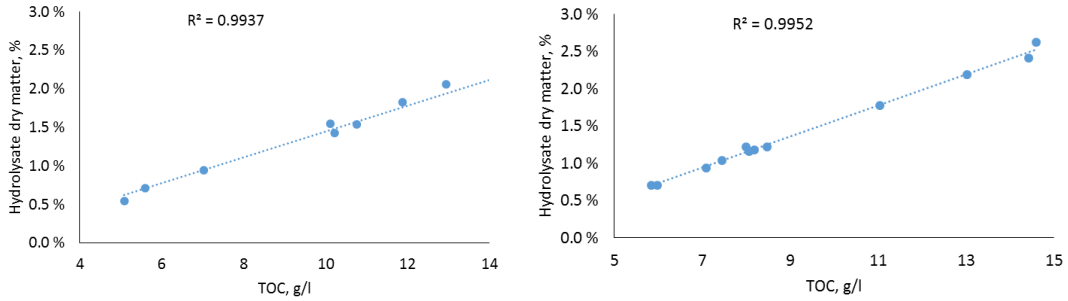


Figure 21 on left and 22 on right. Relationship between TOC and hydrolysate dry matter in euca on left and kraft, oxygen delignified on right.

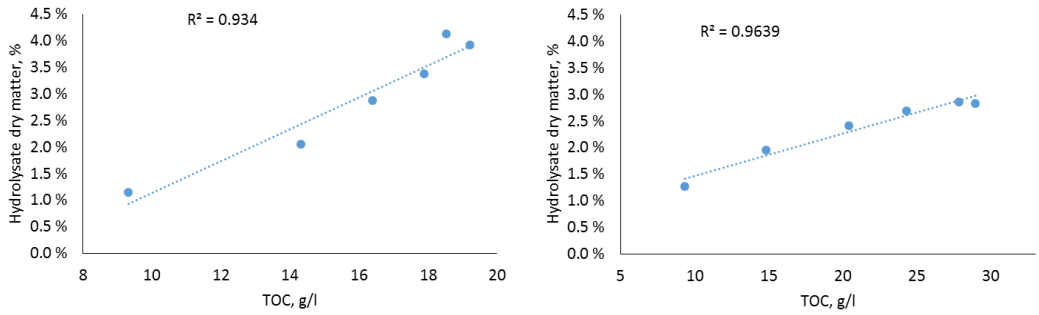


Figure 23 on left and 24 on right. Relationship between TOC and hydrolysate dry matter in kraft, bleached. Titrated on left and constant on right.

3.2.4. Losses due to very fine material accumulation

The washing losses were significant with kraft, bleached. All other results had acceptable errors by estimation with mass balance, but kraft, bleached needed further examination. The losses were measured with reproduction of two batches and from these the results were that losses were equal to uncertainty factor based on mass balance calculations. In essence, all material was accounted for by estimation with mass

balance but the washing losses in kraft, bleached. The losses were suggested prior study to cause inconsistencies in results but the issue was dismissed in the planning as previously this had not been a problem. In other experiments run with similar settings, the intensities used did not produce so much fine material that could exit the wire bags. Mass balance from kraft, bleached, constant is shown in Figure 25.

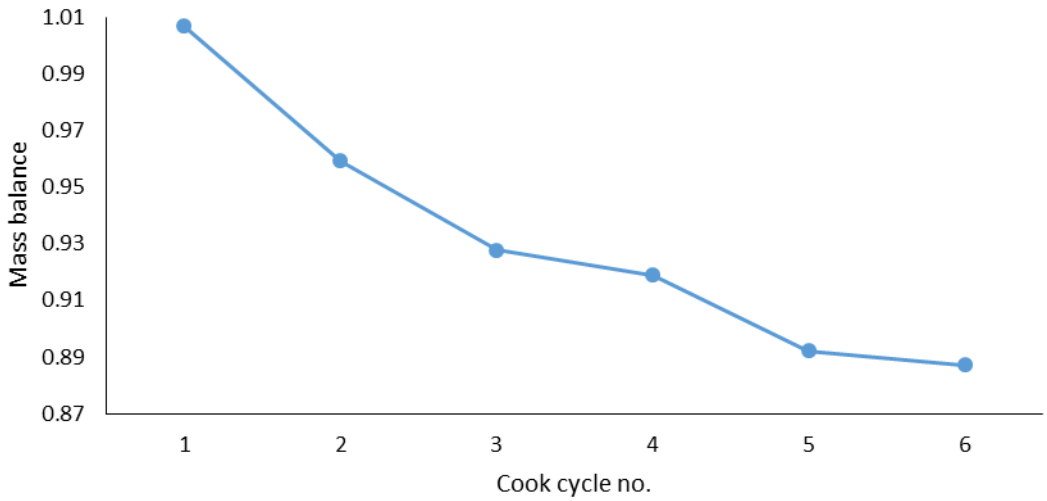


Figure 25. Mass balance as defined in methods from kraft bleached, constant.

The fines material produced can controlled with the same treatment intensity modifications introduced earlier. In process environment, this will not cause trouble as long as the process intensity is controlled.

3.2.5. TOC and humin formation

Almost all of the changes in total sugar and acid concentrations in the constant acid loading settings with euca and kraft, bleached were explained by changes in amounts of TOC-measurable carbon. In other series, it was not as clear, and it might be possible that the unaccounted part was involved in formation of condensated humins. This would

translate into the optimum hydrolysis conditions being on the higher side of intensities tested in this work. The suggested humin formation was strongest in series with kraft, oxygen delignified and the formation of complex polymers with lignin might cause this type of loss in sugar and acid yields. The relationships between TOC and sugar and acid concentrations in series with euca, constant and kraft, bleached, constant are shown in Figures 26 and 27. Almost all of the changes in sugar and acid concentrations are explained by changes in TOC. It is possible that practically all of the carbon compounds in these settings produced either sugars or acids. Similar plotting as was produced for other series but the calculations resulted in lower R^2-values. The unaccounted portion is suggested to be caused by formation of humins. The relationship between TOC and sugar and acid concentrations for other series are presented as R^2-values in Table 3.

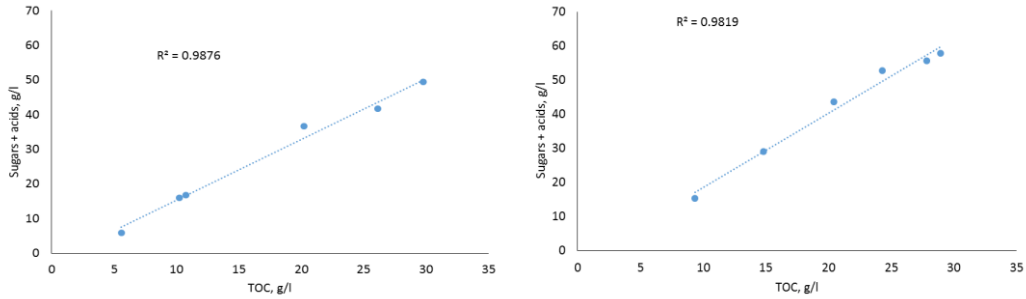


Figure 26 on left and 27 on right. Relationship between TOC and sugar and acid concentrations in euca, constant on left and kraft, bleached, constant on right.

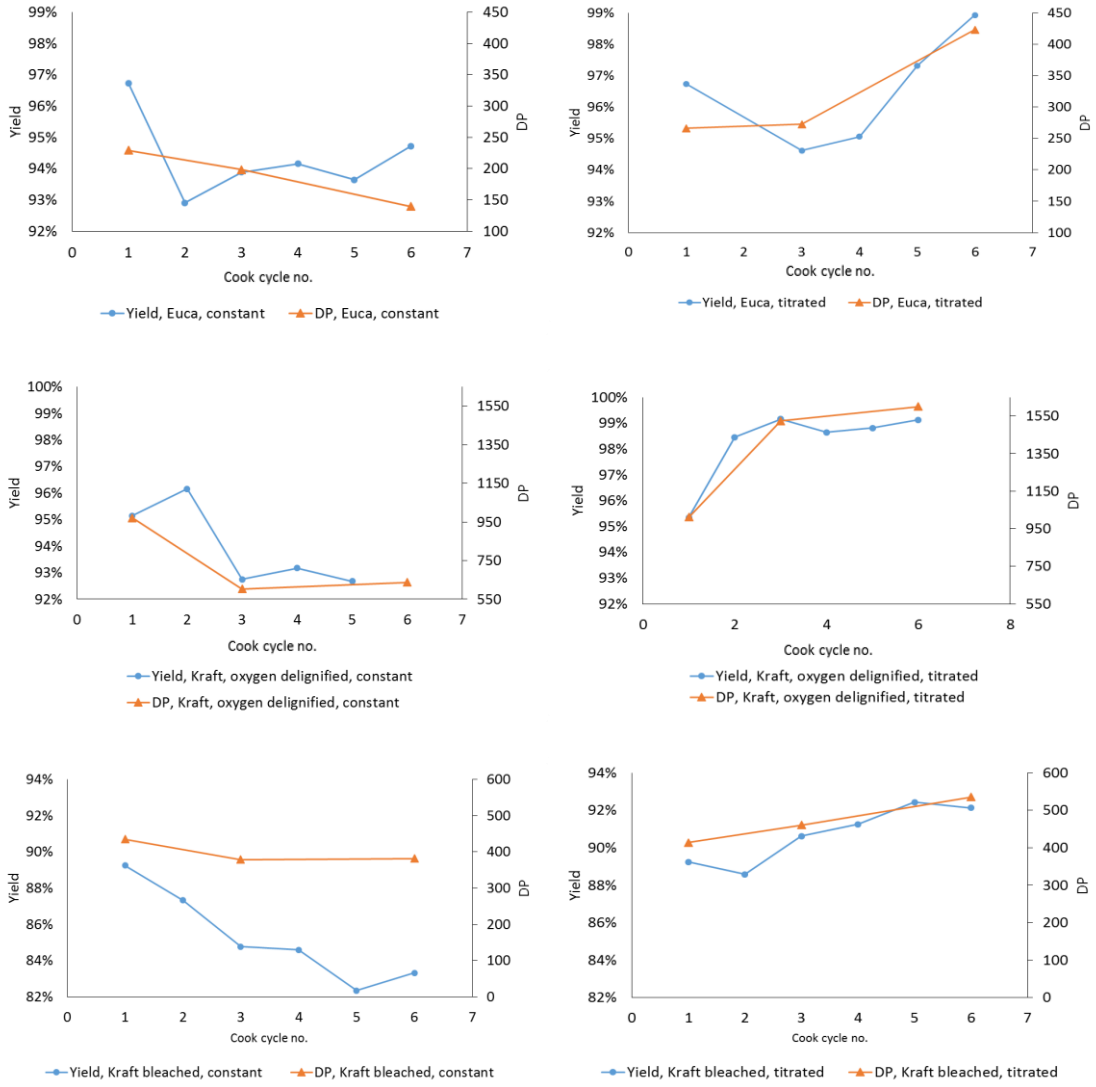
Table 3. Relationship between TOC and sugar and acid concentrations.

Treatment series	**Total sugars + acids plotted against TOC, R2 value of linear regression curve**
Kraft, oxygen delignified, titrated	0.8411
Kraft, oxygen delignified, constant	0.9477
Kraft bleached, titrated	0.9353
Euca, titrated	0.9263

Humin formation was visually examined and in series with kraft, oxygen delignified, titrated it was easy to spot condensated particles especially towards the end of the cooking cycles. In the best performing series with kraft, bleached, constant and euca, constant, there was no signs of humin condensation and in other series small amounts of particles were detected in the hydrolysate after a few days of storage. In double wash, the hydrolysate was clear. The measurement of actual rates of humin formation could not be fitted into timescale of this work.

3.2.6. Yield and DP progression with proceeding cook cycles and role of organic acid formation in the process

The comparison of yield progress in proceeding cook cycles with DP changes solidifies the idea that the treatment intensities change differently with constant and titrated acid loading. In titrated series there is an increasing trend in both DP and yield and in constant series vice versa. The differences are shown in Figures 28–33.



Figures 28, 30 and 32 on left top down, Figures 29, 31 and 33 on right top down. DP and yield progress in proceeding cook cycles. The constant series are shown on left and titrated series on right.

From the DP results it is clear that at these concentrations, the organic acids are not sufficient for adequate hydrolysis. By comparison with results by Kupiainen (2012) at least tenfold increase in organic acid amounts would be needed for production of microcrystalline cellulose with formic acid. Although all organic acids affect the hydrolysis to some degree, it is impossible to estimate the exact impact they have on the hydrolysis process. Even as the weak acids can't hydrolyze the cellulose in these

concentrations, the hydrolysis works with formic acid also. H^+-ion concentration seems to be main controlling factor, but in very low pH the organic acids can't separate. If the hydrolysate consisted mostly of organic acids, they strengthened the hydrolysis in series with constant acid loading significantly. It is also possible that hydrolysate contained unreacted sulfuric acid that affected the intensity with proceeding cook cycles, but it is impossible to separate these effects as the amount of sulfuric acid residue in hydrolysates was not measured.

The DP in double wash seems to behave similarly in regards to yield. In first two cooking cycles the process intensifies a bit and in subsequent treatments the intensity seems to stabilize. In continuous process this translates that the intensity of the treatment may successfully be manipulated by changing the share of hydrolysate stream going back to reactor. Comparison of DP and yield progress in proceeding cook cycles for double wash is shown in Figure 34.

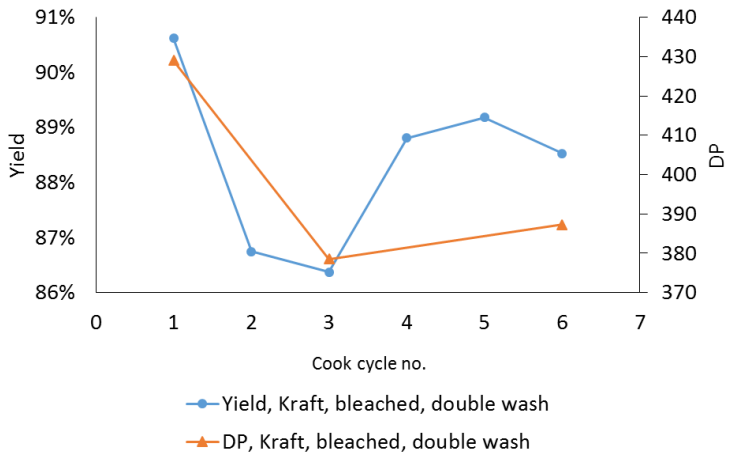


Figure 34. Comparison of DP and yield progress in proceeding cook cycles in double wash.

3.2.7. Produced fiber DP and particle size and related treatment intensity

Particle size was noted to be measuring the DP quite well. In some of the particle size analyses, parts of the material showed to be not hydrolyzed but even so, the relationship between these two values was strong. The relationship is shown in Figure 35.

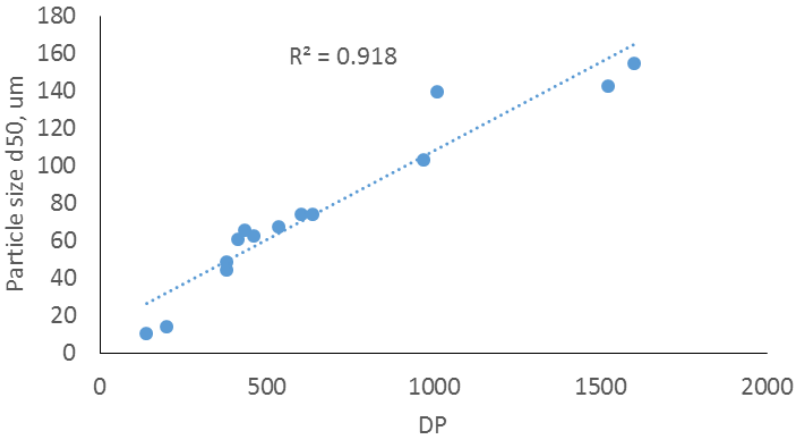
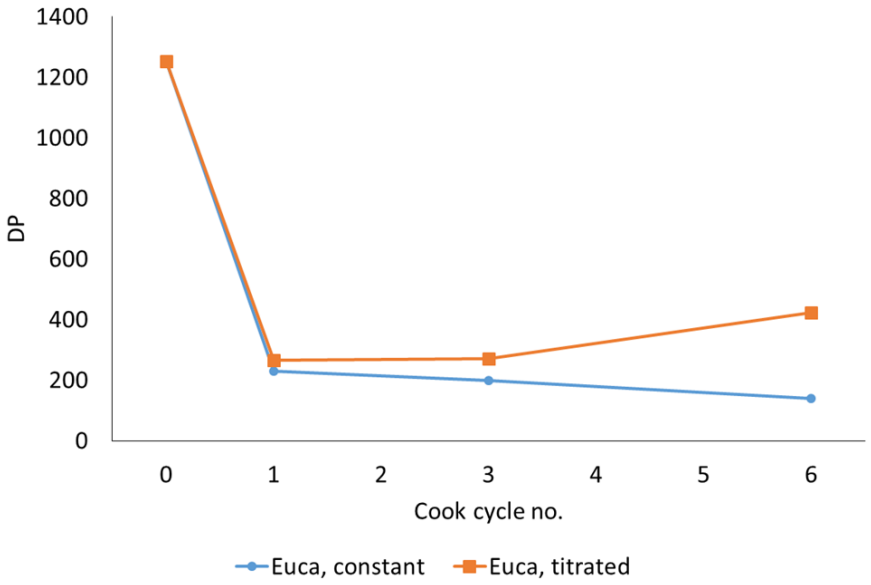


Figure 35. Relationship between particle size and DP. All constant and titrated series are shown in Figure. The linear regression is shown for visual purposes only as the sample size is not sufficient for conclusions on regression.

The hydrolysis produced microcrystalline cellulose in all treatments series with euca, constant, kraft, bleached, constant and double wash. All the other series did not have sufficiently strong treatment intensity to lower the DP down to at least 400. Even with the kraft, bleached and double wash, the first hydrolyses produced a DP of 414 to 435 which could be considered insufficient. The DP progress in proceeding cook cycles is shown in Figures 36–39. Only constant acid loading resulted in production of acceptable microcrystalline cellulose. With kraft, oxygen delignified, no acceptable microcrystalline cellulose was produced by evaluation of DP results.



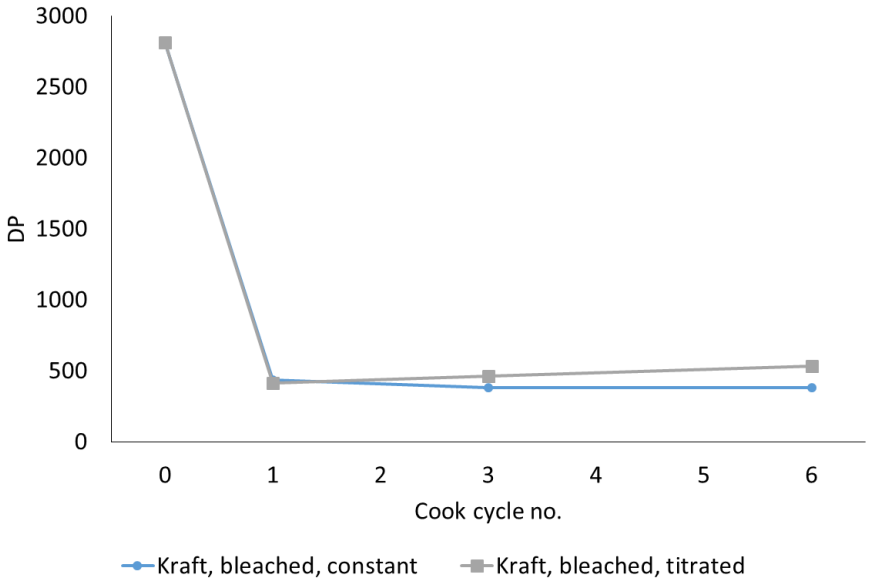


Figure 36 on top and Figure 37 on bottom. The DP progress in proceeding cook cycles in four series with euca and kraft, bleached in both treatments.

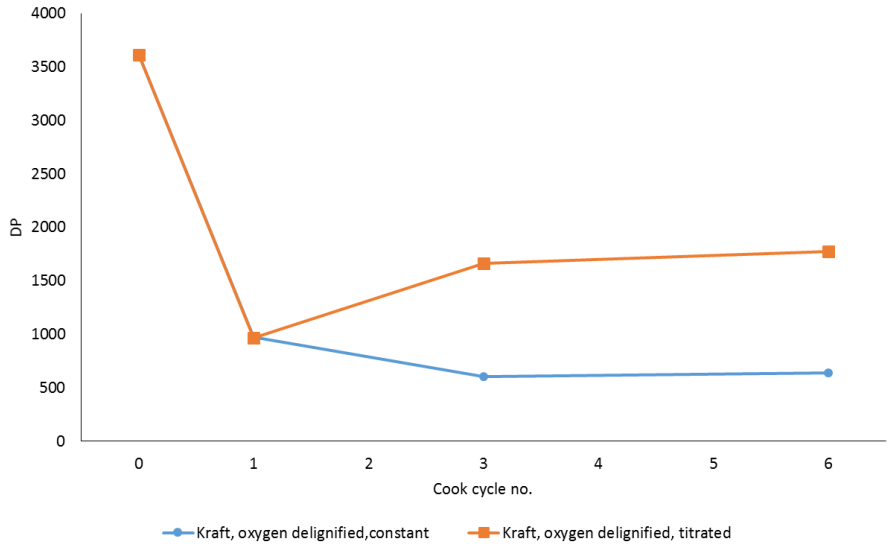


Figure 38. The DP progress in proceeding cook cycles two series with kraft, oxygen delignified in both treatments.

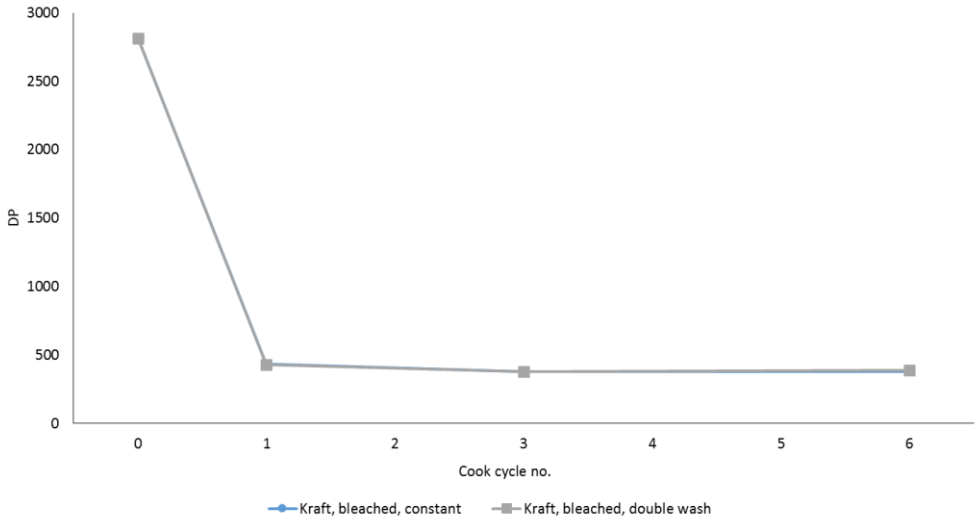


Figure 39. The DP progress in proceeding cook cycles with kraft, bleached in constant acid loading and double wash.

The double wash and constant acid loading resulted in similar viscosities of the microcrystalline cellulose. Characteristically they may differ in particle size distribution,

but the reported measures were close to identical. The measurements of viscosity with kraft, bleached were done with the washed material that had lost much of the fines material so the particle size comparisons would not be meaningful. Results still show that the recycling the hydrolysate in reactor is practical when the intensity of treatment is set to a stable level.

In a production setting, the intensity can be controlled in many ways: the amount of added sulfuric acid to the share of hydrolysate returned to reactor can be adjusted to a level that will produce constant results. Even with constant acid loading, the intensity of treatment can be managed by only controlling the amount of hydrolysate going back to reactor.

3.2.8. Relationship between pH and conductivity

The conductivity of the hydrolysates were measured to be inversely related to pH. This relationship is shown in Figure 40. As pH was only controlling factor in treatment intensity, it is clear that the intensities are getting higher with proceeding hydrolyses in series with constant loading. This can be seen in Figures 42 and 44. The opposite is happening in series with titrated acid loading: as expected from previous results, the pH is lowering with proceeding hydrolysis series and treatment intensities are getting lower. This is shown in Figures 41 and 43. Similar results were seen in measurements of yields and DP. Also stabilization of pH and conductivity are seen in proceeding series with double wash. This is in line with previous statements and shown in Figures 42 and 44.

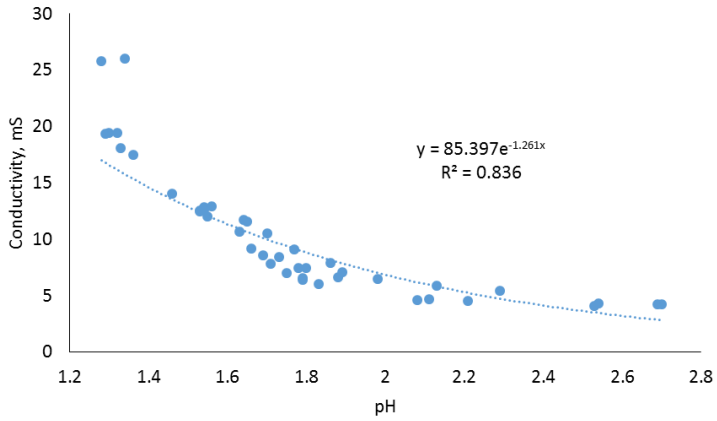
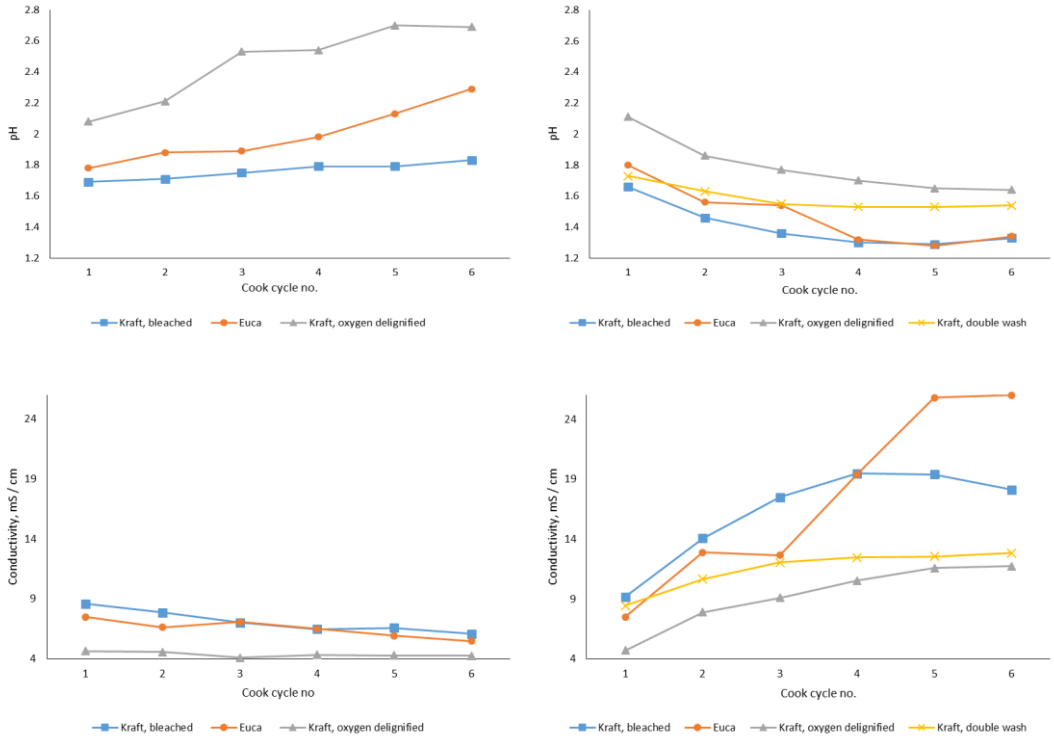


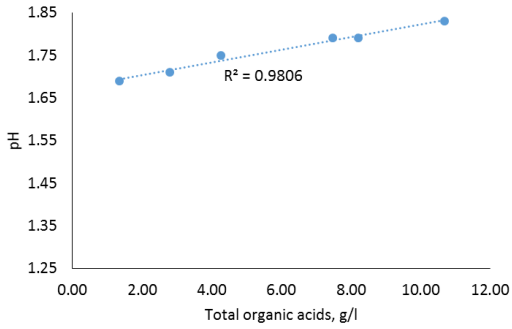
Figure 40. Relationship between conductivity and pH. The equation and trendline are only for demonstration purposes.



Figures 41 and 43 on left top down and Figures 42 and 44 on right top down. Conductivity and pH in proceeding series. The titrated series on left side and constant series and double wash on right.

3.2.9. The origin of the hydrolysate acidity

In a comparison between organic acid amounts and pH, it seems that it might be possible that sulfuric acid residues affect the resulting pH more than the organic acids. Relationships between organic acid content and resulting pH are shown in Figures 45 and 46. The high regression in titrated series can be explained by the acid loading being adjusted by results from titration; the added amount of sulfuric acid was related to organic acid content and so the pH relates linearly to the amount of added sulfuric acid also. The regression value for this addition is very high as shown in Figure 45. From Figure 46, in which relation of pH to organic acids in constant loading is shown, an opposite relation is found. This may be due to pH getting lower in proceeding series and that the relation of increasing organic acid content with pH is incidental and the lower pH values are caused by sulfuric acid addition. While it is possible that the organic acids affect the hydrolysis strongly, it seems that at these concentrations, the sulfuric acid is driving the hydrolysis. The different direction of relation between produced organic acids and treatment pH in titrated and constant series is related to methods but also might be telling about the contribution of different acids to hydrolysis. More research would be needed for conclusions on whether it is sulfuric acid residues or organic acids that affect the hydrolysis intensities most.



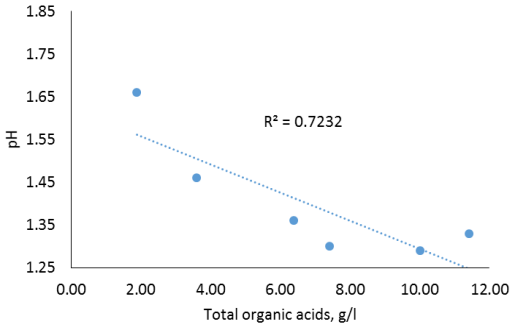
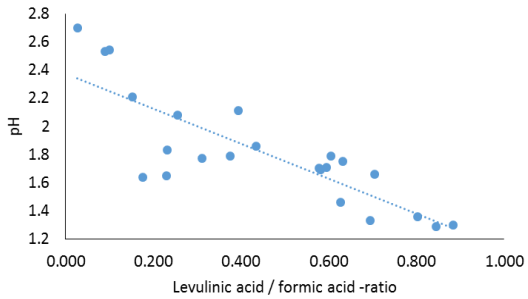


Figure 45 on left and Figure 46 on right. Relationship between pH and organic acid content in kraft, bleached in both treatments. Kraft, bleached, titrated on left and kraft, bleached, constant on right.

3.2.10. The modifications to produce favoured products in the hydrolysate

The pH was also related to produced ratios of levulinic acid to formic acid, and sugars to acids. This may be also related to other factors that participate in treatment intensity but it seems that by modifying the process conditions, certain product formation ratios can be modified. The ratios are shown in Figures 47 and 48. The trends seen in these results could be made clearer if raw materials were subjected to conditions in which the only change in treatment was the pH change. In this setting, all hydrolyses have different amounts of solutes in the hydrolysate added to reactor. The ratio effects were only calculated using data for kraft, bleached and kraft, oxygen delignified. These suggested ratio effects would need extensive testing to produce results that could be used as guidelines but in essence these kind of evaluations are necessary to improve process yields of preferred products. The kraft in double wash was expected to react similarly in terms of described ratios but measurements were omitted from the Figures 47 and 48 as values were not comparable due to only partial usage of hydrolysate as reaction medium.



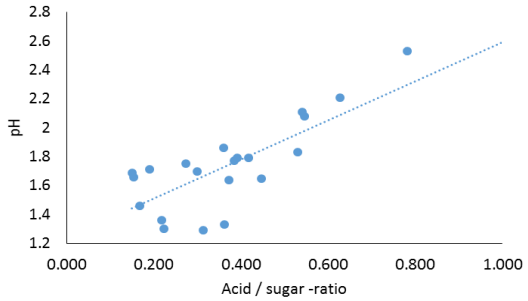


Figure 47 on left and Figure 48 on right. Relationship of pH in hydrolysis to production ratios of levulinic acid to formic acid and of acid to sugar consistency in hydrolysate. The calculation included kraft, bleached and kraft, oxygen delignified.

3.2.11. Sugar and acid analysis results

The results from sugar analysis with HPAE-PAD and acid analysis with HPLC are described in Figures 49–64 in following pages. The production of sugars and acids were strongest in series with kraft, bleached, constant. This can be expected, as in the same series the treatment intensity was highest and reduction in DP significant. At these sugar levels with removal of fermentation inhibitors, Dussan et al. (2014) state the fermentation process economical. The process economics are highly dependent on separation efficiency of sugars and other process factors. Sugar concentrations can also be increased by e.g. filtration techniques. Qi et al. (2014) present a method of nanofiltration to separate increase the glucose concentrations from hydrolysates with similar glucose concentrations. The evaluation of fermentation process economics is mostly out of the scope of this work but in principle, sugars accumulate in the hydrolysate as expected and it might be possible to viably process the sugar produces into alcohols. In the process with most sugars produced, the further degradation of sugars into acids seem to restrict the yield of sugars in latter hydrolyses of the series. This might be problematic in process environment if the sugar concentrations achieved are deemed too low for alcohol fermentation. The double wash is omitted in Figures 49 and 50 as it is not comparable due to removal of part of the sugar and acid content from each hydrolysis.

Some of the integrations of levulinic acid concentrations may include some other acids in Figures 49–64, as the acid peaks in HPLC were inseparable in some results; parallel Figures have differing y-axles.

Membrane separation with nanofiltration and combined electrodialysis could be used for separation of levulinic acid and 5-HMF from the hydrolysate (Kim et al., 2013). At these concentrations it is not clear whether membrane fouling or other process issues would affect the process. Mainly the processes that aim at levulinic acid production consider converting as much of glucose as possible to levulinic acid and often aim at degrading the whole raw material (Morone et al., 2015; Bozell et al., 2000). The possibilities of levulinic acid production are generally under evaluation and related patents are processed as the ideas for new biorefinery products are sought. It is hard to evaluate whether the levulinic acid production would be economical from this type of hydrolysis process.

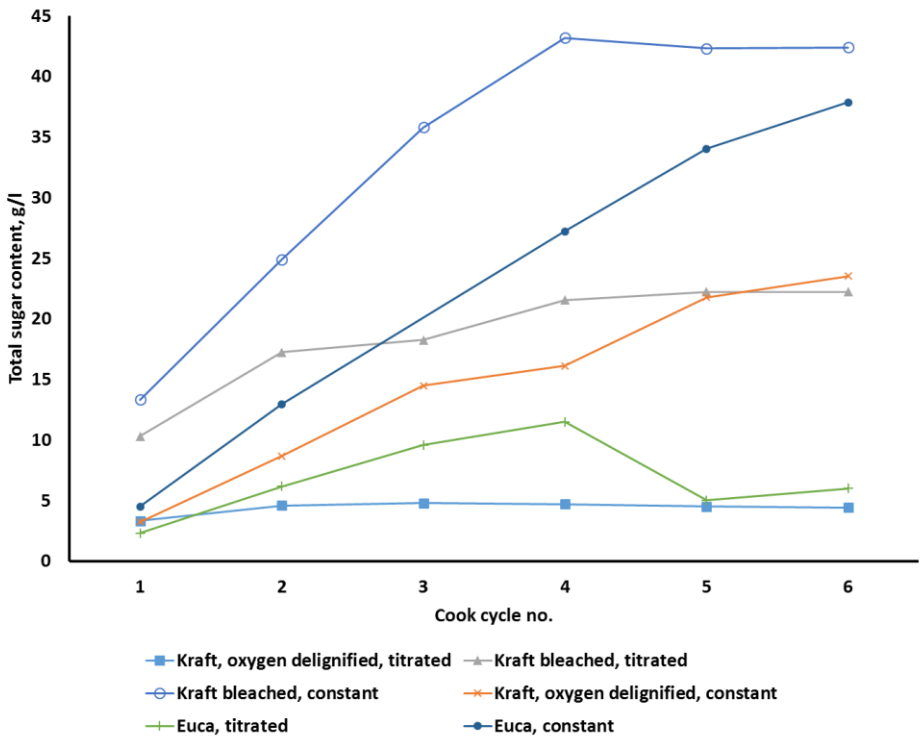


Figure 49. The total sugar contents of all series except double wash.

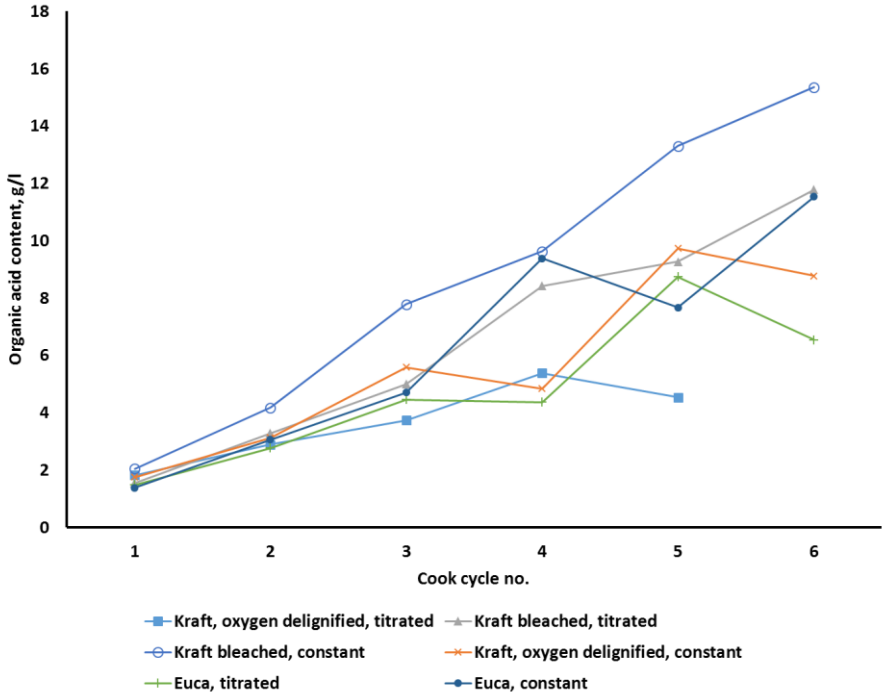


Figure 50. The total organic acid contents of all series except double wash.

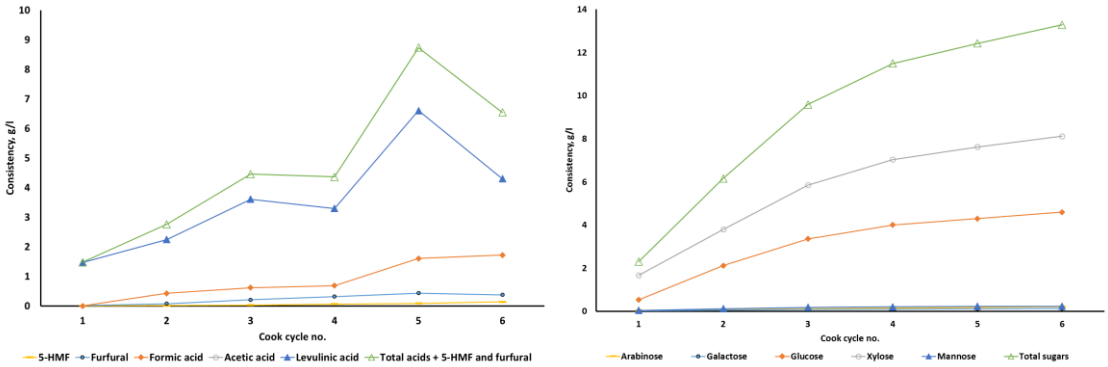


Figure 51 on left and Figure 52 on right. Sugar and acid analysis results of euca, titrated.

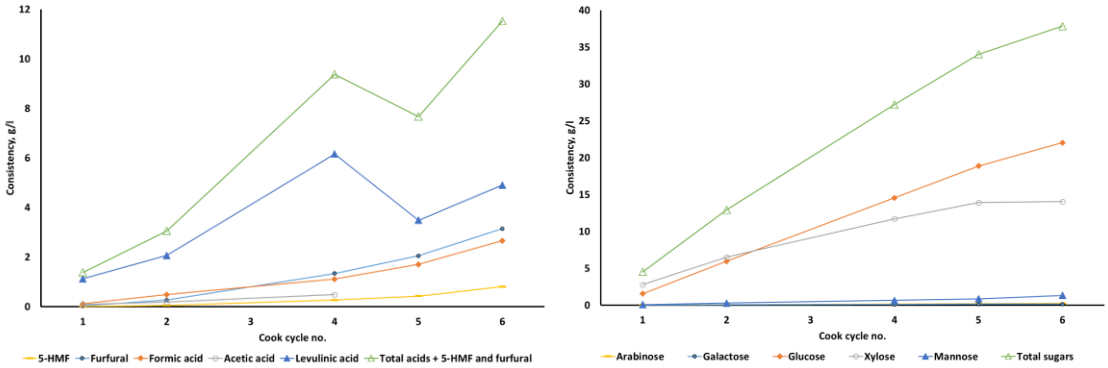


Figure 53 on left and Figure 54 on right. Sugar and acid analysis results of euca, constant.

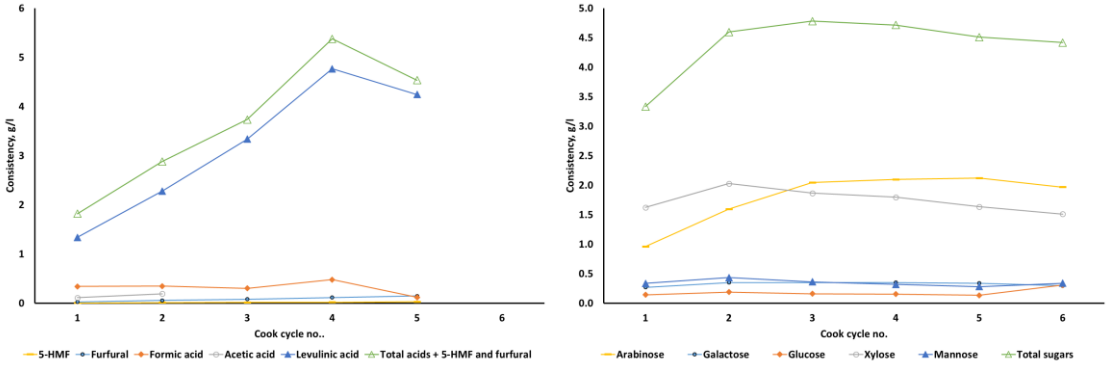


Figure 55 on left and Figure 56 on right. Sugar and acid analysis results of kraft, oxygen delignified, titrated.

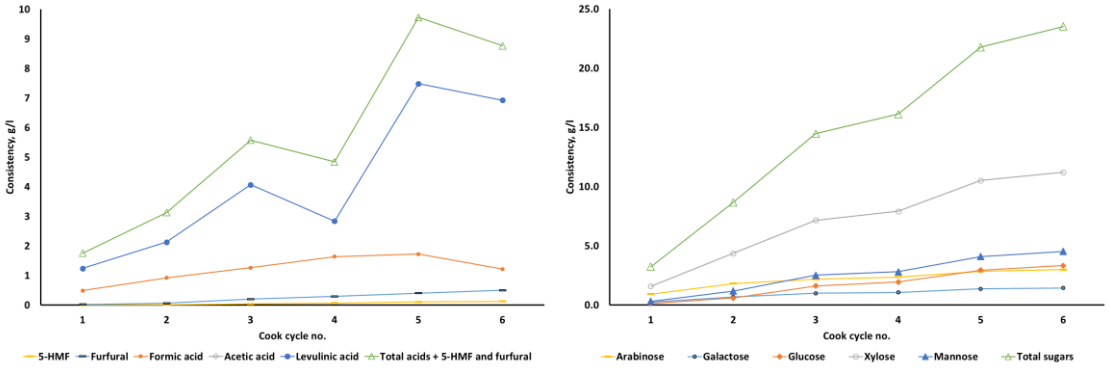


Figure 57 on left and Figure 58 on right. Sugar and acid analysis results of kraft, oxygen delignified, constant.

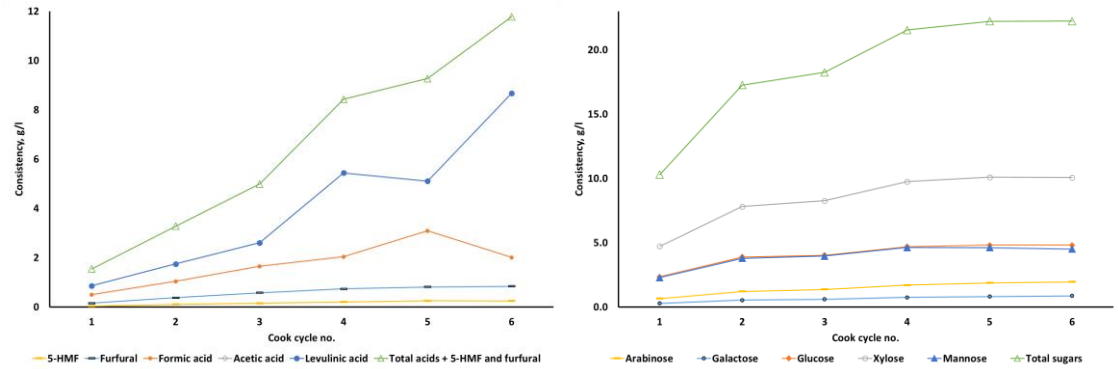
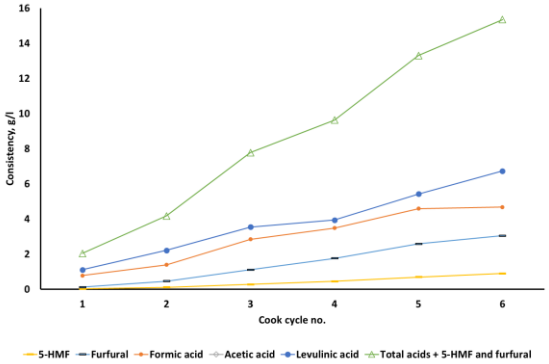


Figure 59 on left and Figure 60 on right. Sugar and acid analysis results of kraft, bleached, titrated.



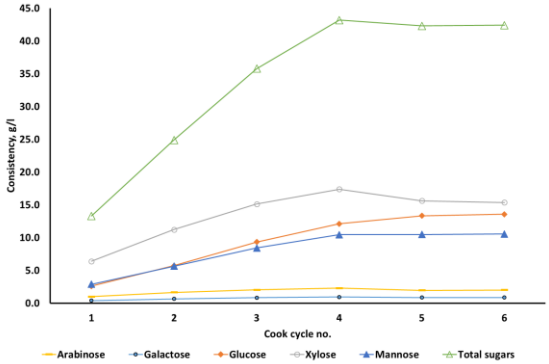
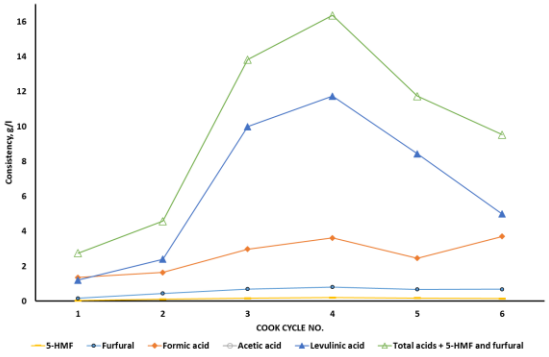


Figure 61 on left and Figure 62 on right. Sugar and acid analysis results of kraft, bleached, constant.



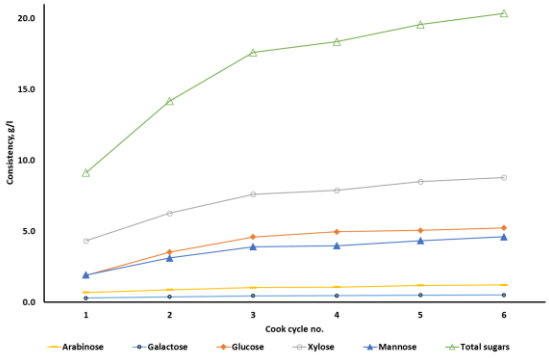


Figure 63 on left and Figure 64 on right. Sugar and acid analysis results of kraft, double wash.

The results from sugar and acid analyses were in line with the expectations from yield and DP changes. Mass balance evaluations based on the sugar and acid consistencies showed similar direction of change as mass balance calculations with hydrolysate dry matter contents used for evaluation. This leads us to expect that these are major reaction pathways in these hydrolysis processes and the production of humic substances or oligomeric sugars have less of a role.

Oligomeric sugars were not measured against standards in HPLC measurements, which could lower the sugar yields to some degree. Most of the oligosaccharides are expected to be degraded into monosaccharides or their derivatives in temperatures of

microcrystalline cellulose production temperatures, but at lower temperatures and very short reaction times the process could produce 10 wt.% of oligosaccharides (Saeed et al., 2012; Garlock et al., 2011; Kupiainen, 2012). In some hydrolyses, the oligosaccharide concentration may have contributed to some part of mass accounted in uncertainty factor.

3.2.12. The brightness of the produced fibers

The brightness of the produced microcrystalline celluloses was lowered significantly with proceeding cook cycles, especially in cases that produced a lot of sugars. As the coloring is in most cases not from lignin contents, but caramelized sugars, it could be easy to bleach the materials. In optimized conditions, the sugar caramelization could be limited if sugar concentrations are lower. The double wash produced significantly brighter microcrystalline cellulose than kraft, bleached, constant and partial removal of sugars should result in brighter material. The ISO-brightness values are shown in Figures 65–68.

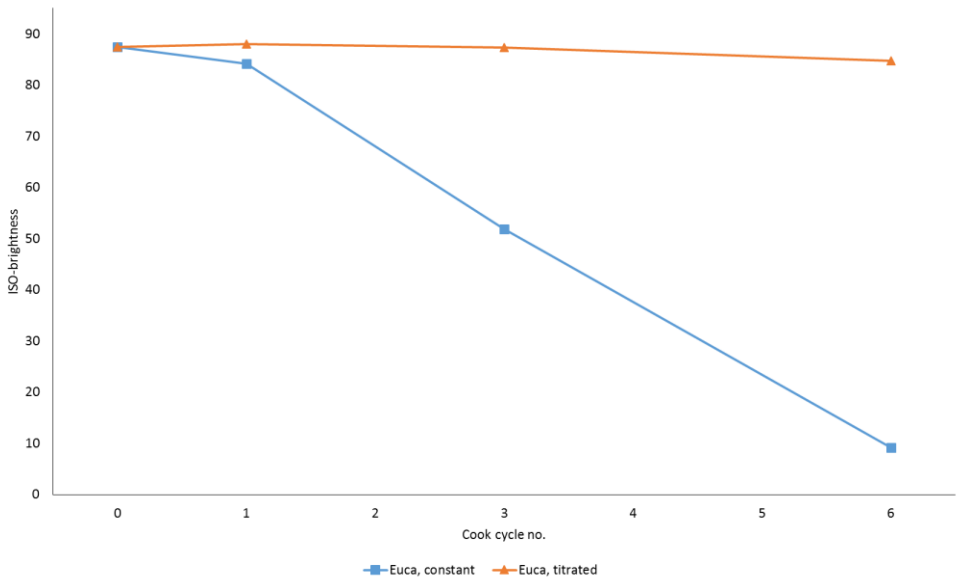


Figure 65. ISO-brightness development of series with euca as raw material.

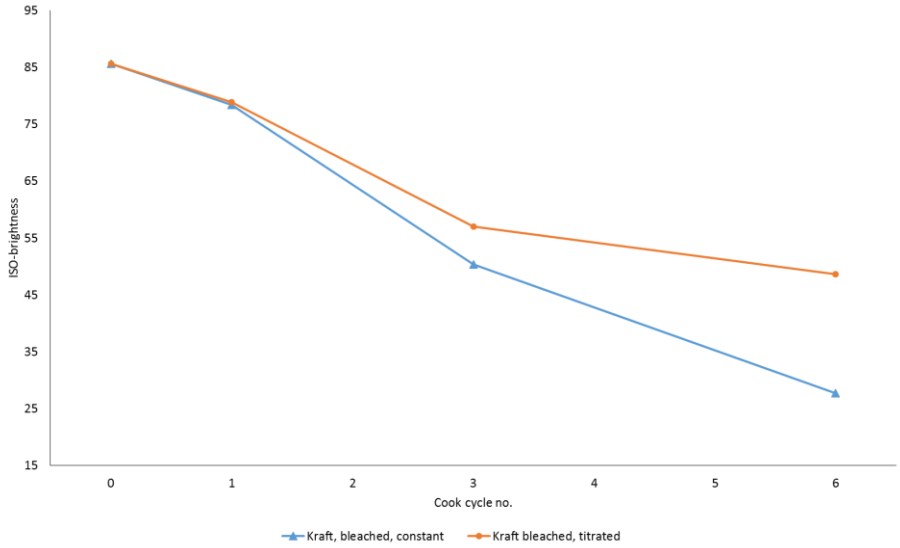


Figure 66. ISO-brightness development of series with kraft, bleached as raw material.

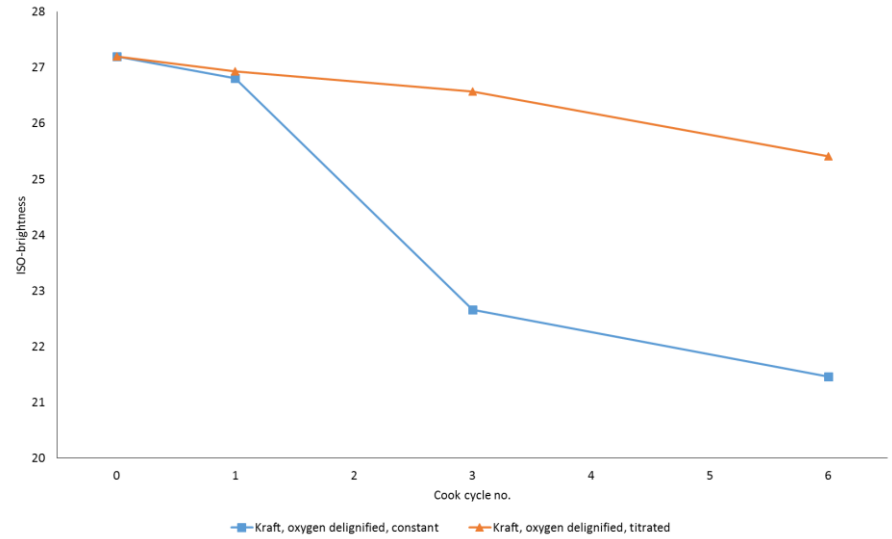


Figure 67. ISO-brightness development of series with kraft, oxygen delignified as raw material.

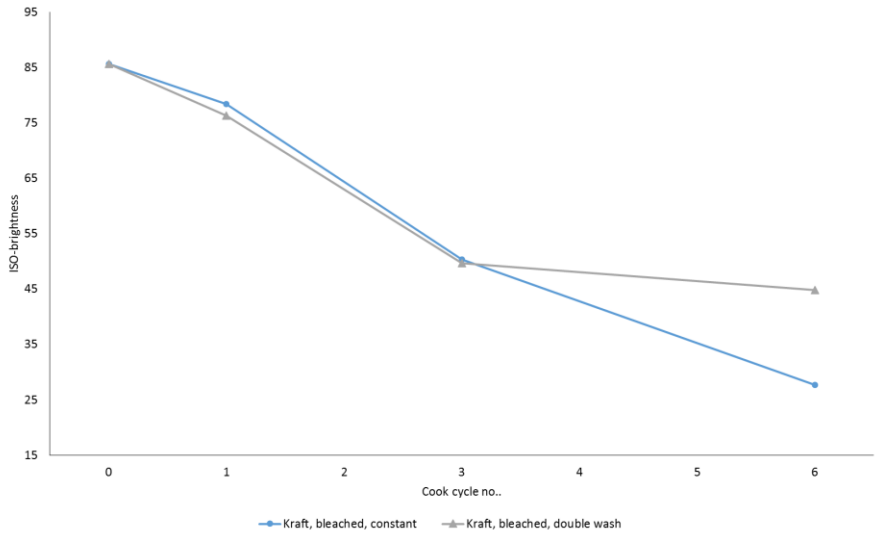


Figure 68. ISO-brightness development of kraft, bleached, constant compared to double wash.

3.2.13. Effects of treatment to crystallinity of produced fiber

The XRD-analysis showed that the changes in crystallinities due to treatment were between 2 and 4% in all series. The change was towards higher crystallinity, but the changes were low. As microcrystalline cellulose production involves removal of amorphous domains, increase of crystallinity as percentage of resulted material can occur. In some cases, also downward shift of crystallinity can be detected due to hydrolysis to microcrystalline cellulose (Gurgel et al., 2012; Virtanen et al., 2011). The Peak height evaluation method of XRD was used as it gives reasonably accurate estimate of whether the crystallinity is changing significantly in the hydrolysis series although values may not be directly comparable to literature results. The calculated values for crystallinity indices are given in Appendix 2.

3.2.14. Acidic groups in fiber surface

The results from conductometric titration showed no changes in amounts of strongly or weakly acidic groups on fiber surface. The amount of sulfonation was evaluated by results from CHNS-analysis. CHNS-analysis showed that changes in sulfur contents were low: in series with kraft, bleached, constant acid loading the change was at maximum 0.02 percentage points in sulfur content and 0.03 percentage points with euca. The results from series with oxygen delignified, constant acid loading seemed inconsistent: the measurements from first hydrolysis were 0.19 percentage points higher than raw material sulfur contents but measurements from the last hydrolysis only 0.05 percentage points higher. More focus on this phenomena should be made to draw conclusions of the effects of sulfonation amounts in dilute acid treatments of cellulose. Results of the CHNS measurements are included in Appendix 2. It seems that in dilute acid hydrolysis the amount of sulfur in fiber does not change significantly. The sulfonic group formation may be related to microcrystalline cellulose forming stable chiral nematic phase, but the suggested optimum reaction conditions for sulfonation are high concentration acid treatment and low temperature (Rajalaxmi et al., 2010, Dong et al., 1998).

3.2.15. Further considerations of process aspects

If the produced sugar levels are acceptable for example to biogas production, all or most of the water produced from first centrifuge may be directed towards this process. In this case, more water from washing stage can be directed to the reactor. In conditions in double wash, the produced microcrystalline cellulose would also need further processing to achieve high brightness values.

Treatment of oxygen delignified pulp consumes higher amount of acids. Also, it produced materials that caused problems in the measurements. These materials were assumed to be degradation products and humins. The humin formation should be limited if optimum process conditions are met. In treatments with constant acid loading, the humin formation seemed less of an issue. The hydrolysate properties are affected by CSF, but in all cases the 5-hydroxymethylfurfural decomposes quickly in the reaction conditions used in this work.

4. Conclusions

A continuous process can produce acceptable microcrystalline cellulose when proper process controls are in use. If water from first centrifugation is used undiluted as the reaction medium, the ISO brightness of the product is significantly lowered in first few rounds already to unacceptable levels, especially with kraft pulps. The more bright dissolving pulp retained higher brightness after the treatment. Acceptable DP and particle size can be maintained by management of the water flows also in a continuous process.

The amount of sulfuric acid has much more effect on the hydrolysis than organic acids at these concentrations. The organic acids produce titratable H^+-ions but can't lower the pH low enough to drive the hydrolysis. Intensities of the treatments can be effectively estimated by combined severity factor. The pH of the hydrolysis liquid was the defining factor for treatment intensity in this work as other factors in CSF were same in each treatment.

Concentrations of organic acid produced in the hydrolysis were quite low. To separate e.g. levulinic acid much higher concentrations are suggested in current literature. Methods for membrane separation exist but the viability of these are hard to estimate in treatments involved in this work.

Sugar concentrations do not become very high as the degradation processes to organic acids limit the sugar yields. The results hint that the accumulation of sugars into the hydrolysate may diminish as the sugar concentrations increase. Sugar degradation leads to lower ISO-values of produced materials. The browning due to sugar degradation is expected to be easily manageable by addition of a bleaching stage if necessary.

The double wash –treatment may be used to solve some of the issues in hydrolysate circulation. When 35.7% of the produced hydrolysate was returned to reactor, the process seemed to stabilize in proceeding cook cycles. The hydrolysis intensity did not change significantly after second or third hydrolysis. The treatment intensity should be possible to manipulate to some extent with this type of treatment by changing the share of hydrolysate returned to reactor.

In future work it should be estimated if the sugar and acid concentrations allow further processing. The optimization of the continuous process should be started with the process setting described as double wash.

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6. Appendices

Appendix 1

Cook cycle	Sample Name	TOC	TC	IC	YIELD	UNCERTAINTY FACTOR	FILTRATE DRY MATTER	SUGAR CONTENTS						Acid contents											
1000																									
		Arabinose	Galactose	Glucose	Xylose	Mannose	**Total sugars**	5-HMF	Furfural	Formic acid	Acetic acid	Levulinic acid	**Total acids + 5-HMF and furfural**	pH	conductivity	Brightness	Limiting viscosity number [η]	Degree of polymerization	Particle size distribution d(0.5)						
		mg/L	mg/L	mg/L	%		%	g/l	g/l	g/l	g/l	g/l	g/l	g/l	g/l	g/l	g/l	g/l	g/l			mS/cm		ml/g		um
0	Et0																						87.4	515.8	1253.5	54.591
1	Et1	5080	5087	7.319	96.7 %	0.016	0.0054	0.0	0.0	0.5	1.7	0.0	2.3	0.0	0.0	0.0		1.5	1.5	1.78	7.47	88.0	111.7	265.9	31.16
2	Et2	7033	7040	7.343		0.072	0.0095	0.1	0.0	2.1	3.8	0.1	6.2	0.0	0.1	0.4		2.2	2.8	1.88	6.63				
3	Et3	10124	10131	7.245	94.6 %	0.001	0.0155	0.1	0.1	3.4	5.9	0.2	9.6	0.0	0.2	0.6		3.6	4.5	1.89	7.07	87.3	114.3	272.2	31.799
4	Et4	11887	11895	7.914	95.1 %	0.023	0.0183	0.1	0.1	4.0	7.0	0.2	11.5	0.1	0.3	0.7		3.3	4.4	1.98	6.48				
5	Et5	12941	12948	6.884	97.3 %	0.006	0.0206	0.2	0.1	4.3	7.6	0.2	12.4	0.1	0.4	1.6		6.6	8.7	2.13	5.91				
6	Et6	15223	15230	7.232	98.9 %	0.016	0.0234	0.2	0.1	4.6	8.1	0.2	13.3	0.1	0.4	1.7		4.3	6.5	2.29	5.46	84.7	177.7	423	50.979
	Ev0																						87.4	515.8	1254	54.591
1	Ev1	5595	5604	8.894		0.052	0.0071	0.0	0.0	1.6	2.8	0.1	4.5	0.0	0.0	0.1	0.1	1.1	1.4	1.8	7.49	84.2	96.3	229	25.933
2	Ev2	10225	10233	7.659	92.9 %	0.029	0.0143	0.1	0.1	6.0	6.5	0.3	13.0	0.1	0.3	0.5	0.2	2.1	3.1	1.56	12.9				
3	Ev3	10771	10778	7.466	93.9 %	0.123	0.0154													1.54	12.6	51.9	83.3	198	14.413
4	Ev4	20211	20219	7.752	94.2 %	0.086	0.0314	0.2	0.1	14.6	11.7	0.7	27.2	0.3	1.3	1.1	0.5	6.2	9.4	1.32	19.4				
5	Ev5	26135	26142	7.725	93.6 %	0.007	0.0390	0.2	0.1	18.9	13.9	0.9	34.0	0.4	2.1	1.7		3.5	7.7	1.28	25.8				
6	Ev6	29799	29808	9.489	94.7 %	0.040	0.0491	0.2	0.2	22.1	14.1	1.3	37.9	0.8	3.1	2.7		4.9	11.5	1.34	26	9.1	58.5	139	10.545
	Ot0																					27.2	1153.5	3614	94.567
1	Ot1	5983	5991	7.574	95.3 %	0.017	0.0071	1.0	0.3	0.1	1.6	0.3	3.3	0.0	0.0	0.3	0.1	1.3	1.8	2.08	4.62	26.9	424.5	970	139.795
2	Ot2	7098	7106	7.828	98.5 %	0.021	0.0094	1.6	0.4	0.2	2.0	0.4	4.6	0.0	0.1	0.3	0.2	2.3	2.9	2.21	4.55				
3	Ot3	7445	7453	7.422	99.2 %	0.021	0.0104	2.0	0.3	0.2	1.9	0.4	4.8	0.0	0.1	0.3		3.3	3.7	2.53	4.08	26.6	639.8	1664	142.667
4	Ot4	8064	8072	7.439	98.6 %	0.020	0.0116	2.1	0.4	0.2	1.8	0.3	4.7	0.0	0.1	0.5		4.8	5.4	2.54	4.32				
5	Ot5	8187	8195	7.737	98.8 %	0.016	0.0118	2.1	0.3	0.1	1.6	0.3	4.5	0.0	0.1	0.1		4.2	4.5	2.7	4.27				
6	Ot6	8477	8484	7.643	99.1 %	0.021	0.0122	2.0	0.3	0.3	1.5	0.3	4.4							2.69	4.25	25.4	672.3	1776	154.615
	Ov0																					27.2	1153.5	3614	94.567
1	Ov1	5842	5853	11.42	95.1 %	0.015	0.0070	0.9	0.3	0.1	1.6	0.3	3.2	0.0	0.0	0.5		1.2	1.8	2.11	4.69	26.8	407.8	971	103.397
2	Ov2	8007	8014	7.392	96.2 %	0.024	0.0122	1.8	0.7	0.6	4.4	1.2	8.7	0.0	0.1	0.9		2.1	3.1	1.86	7.88				
3	Ov3	11036	11043	7.427	92.7 %	0.004	0.0178	2.2	1.0	1.6	7.2	2.5	14.5	0.0	0.2	1.3		4.1	5.6	1.77	9.09	22.7	253.5	604	73.956
4	Ov4	13025	13033	7.295	93.2 %	0.008	0.0219	2.4	1.1	1.9	7.9	2.8	16.1	0.1	0.3	1.6		2.8	4.8	1.7	10.5				
5	Ov5	14413	14421	7.909	92.7 %	0.005	0.0241	2.8	1.4	2.9	10.5	4.1	21.8	0.1	0.4	1.7		7.5	9.7	1.65	11.6				
6	Ov6	14599	14606	7.403		0.092	0.0262	3.0	1.4	3.3	11.2	4.5	23.5	0.1	0.5	1.2		6.9	8.8	1.64	11.7	21.5	267.5	637	74.449
	Bt0																					85.6	953.0	2811	101.672
1	Bt1	9317	9324	7.437	89.2 %	0.127	0.0115	0.7	0.3	2.3	4.7	2.3	10.3	0.0	0.2	0.5		0.9	1.6	1.69	8.58	78.9	173.8	414	60.771
2	Bt2	14315	14323	7.45	88.6 %	0.010	0.0205	1.2	0.5	3.9	7.8	3.8	17.3	0.1	0.4	1.0		1.8	3.3	1.71	7.84				
3	Bt3	16391	16399	7.568	90.6 %	0.021	0.0288	1.4	0.6	4.0	8.3	4.0	18.2	0.2	0.6	1.6		2.6	5.0	1.75	7	57.0	193.3	460	62.444
4	Bt4	17883	17890	7.384	91.3 %	0.015	0.0338	1.7	0.8	4.7	9.8	4.6	21.5	0.2	0.7	2.0		5.4	8.4	1.79	6.45				
5	Bt5	19226	19235	8.816	92.4 %	0.039	0.0392	1.9	0.8	4.8	10.1	4.6	22.2	0.2	0.8	3.1		5.1	9.3	1.79	6.57				
6	Bt6	18525	18532	7.776	92.1 %	0.018	0.0414	2.0	0.9	4.8	10.1	4.5	22.2	0.2	0.8	2.0		8.7	11.8	1.83	6.07	48.6	224.7	535	67.535
	Bv0																					85.6	953.0	2811	101.672
1	Bv1	9351	9359	7.534	89.2 %	0.007	0.0127	1.0	0.4	2.6	6.4	2.9	13.3	0.0	0.1	0.8		1.1	2.0	1.66	9.17	78.4	182.5	435	65.696
2	Bv2	14833	14840	7.666	87.3 %	0.041	0.0195	1.6	0.7	5.7	11.2	5.6	24.9	0.1	0.5	1.4		2.2	4.2	1.46	14.1				
3	Bv3	20434	20441	7.668	84.8 %	0.072	0.0241	2.0	0.8	9.3	15.1	8.4	35.8	0.3	1.1	2.8		3.5	7.8	1.36	17.5	50.3	159.0	379	48.952
4	Bv4	24298	24305	7.608	84.6 %	0.081	0.0269	2.3	0.9	12.1	17.4	10.5	43.2	0.5	1.8	3.5		3.9	9.6	1.3	19.5				
5	Bv5	27832	27840	7.828	82.3 %	0.108	0.0287	2.0	0.9	13.3	15.6	10.5	42.3	0.7	2.6	4.6		5.4	13.3	1.29	19.4				
6	Bv6	28964	28973	9.435	83.3 %	0.113	0.0284	2.0	0.9	13.6	15.4	10.6	42.4	0.9	3.0	4.7		6.7	15.4	1.33	18.1	27.7	160.2	381	44.767
																						85.6		2811	
1	T1				90.6 %		0.9 %	0.7	0.3	1.9	4.3	1.9	9.1	0.0	0.2	1.4		1.2	2.7	1.73	8.4	76.3	180.2	429	
2	T2				86.7 %		1.4 %	0.9	0.4	3.5	6.3	3.1	14.2	0.1	0.4	1.6		2.4	4.6	1.63	10.7				
3	T3				86.4 %		1.7 %	1.0	0.4	4.6	7.6	3.9	17.6	0.2	0.7	3.0		10.0	13.8	1.55	12.0	49.6	159	379	
4	T4				88.8 %		1.7 %	1.1	0.5	5.0	7.9	4.0	18.3	0.2	0.8	3.6		11.7	16.4	1.53	12.5				
5	T5				89.2 %		1.8 %	1.2	0.5	5.1	8.5	4.3	19.6	0.2	0.7	2.5		8.4	11.7	1.53	12.5				
6	T6				88.5 %		1.8 %	1.2	0.5	5.2	8.8	4.6	20.4	0.1	0.7	3.7		5.0	9.5	1.54	12.8	44.8	162.7	387	

Sample name codes listed in Appendix 2.

Appendix 2.

Sample name	Br	Bt-1	Bt-6	Bv-1	Bv-6	Or	Ot-1	Ot-6	Ov-1	Ov-6	Er	Et-1	Et-6	Ev-1	Ev-6
Carbon	42.46 %	42.66 %	42.69 %	42.58 %	42.63 %	42.63 %	43.19 %	43.11 %	43.07 %	43.19 %	42.50 %	42.69 %	42.61 %	42.80 %	42.58 %
Hydrogen	6.66 %	6.68 %	6.62 %	6.63 %	6.61 %	6.48 %	6.74 %	6.61 %	6.44 %	6.39 %	6.30 %	6.50 %	6.44 %	6.42 %	6.49 %
Nitrogen	0.14 %	0.11 %	0.10 %	0.19 %	0.17 %	0.15 %	0.12 %	0.19 %	0.32 %	0.31 %	0.53 %	0.30 %	0.36 %	0.32 %	0.36 %
Sulfur	0.66 %	0.64 %	0.66 %	0.65 %	0.65 %	0.65 %	0.84 %	0.70 %	0.51 %	0.49 %	0.51 %	0.52 %	0.53 %	0.54 %	0.53 %
XRD (peak height method)	86.6 %			87.2 %	88.3 %	84.2 %			86.4 %	86.5 %	86.0 %			90.3 %	88.7 %
XRD (deconvolution method)	90 %			91 %	92 %				91 %	91 %	91 %			92 %	91 %

Name coding for samples	
First letter is type of pulp	
B	Kraft, bleached
O	Kraft, oxygen delignified
E	Euca
T	Double wash, kraft bleached
Second letter is type of treatment	
t	titrated acid loading
v	constant acid loading
Number is cook cycle number	